

## Research Article

# In Vitro Biocompatibility of Endodontic Sealers Incorporating Antibacterial Nanoparticles

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The main cause of endodontic disease is bacteria. Disinfection is presently achieved by cleaning the root canal system prior to obturation. Following setting, root canal filling is devoid of any antibacterial effect. Endodontic sealers with antimicrobial properties yet biocompatible may enhance root canal therapy. For this purpose, quaternized polyethylenimine nanoparticles which are antibacterial polymers, biocompatible, nonvolatile, and stable may be used. The aim of the present study was to examine the impact of added QPEI on the cytotoxicity of AH Plus, Epiphany, and GuttaFlow endodontic sealers. The effect of these sealers on the proliferation of RAW 264.7 macrophage and L-929 fibroblast cell lines and on the production of TNF $\alpha$  from macrophages was examined. Cell vitality was evaluated using a colorimetric XTT assay. The presence of cytokines was determined by two-site ELISA. Results show that QPEI at 1% concentration does not impair the basic properties of the examined sealers in both macrophages and fibroblast cell lines. Incorporation of 1% QPEI into the sealers did not impair their biocompatibility. QPEI is a potential clinical candidate to improve antibacterial activity of sealers without increasing cytotoxicity.

## 1. Introduction

Endodontic disease of human teeth, a common reality, is caused primarily by bacteria [1, 2]. State-of-the-art disinfection is achieved through biomechanical preparation that includes mechanical instrumentation and antiseptic irrigation. Shaping the root canal space allows for a three-dimensional obturation and seal of the root canal system. The endodontic sealer coats the walls of the canals and fills the space between the root canal filling material and the root, thus filling the root canal and sealing its ports of entry and exit. The set root canal filling material is mostly inert. However, it is expected to physically prevent oral bacterial contamination as well as regrowth of residual bacterial contamination thus protecting the tooth supporting tissues [3, 4]. Regrettably, even when effective instrumentation, irrigation, and an adequate obturation of the root canal are

performed the irregularity in shape (lateral ducts, and anastomosis) allows for bacterial persistence. In most cases, failure of endodontic treatment is associated to the persistence of bacteria in the root canal system [5] or their consecutive coronal leakage. Consequently, bacterial eradication from the root canal system and prevention of coronal leakage are of utter importance. Therefore, root canal sealers with good sealing ability and longer antimicrobial activity are desirable. Moreover, antibacterial endodontic sealers should be lethal against contaminating bacteria without harming tooth-supporting tissues [6].

Research has shown a positive correlation between antibacterial properties of sealers and their cytotoxic effect [7]. Cytotoxicity becomes even more relevant since extrusion of sealer during root canal obturation is a common clinical finding [8]. The cytotoxic effect depends on the leachability of the material [9]. Root canal filling materials

TABLE 1

Material	Composition		Manufacturer
	Paste A	Paste B	
AH Plus	(i) Diglycidil-bisphenol-A-ether. (ii) Calcium. (iii) Tungsten. (iv) Zirconium oxide. (v) Aerosol. (vi) Iron. (vii) Oxide.	(i) Amina 1-adamantane. (ii) N,N dibenzyl-5-oxanonandiamine-1,9. (iii) TCD-diamine. (iv) Calcium tungsten. (v) Zirconium oxide. (vi) Silicone oxide.	AH Plus Dentsply/Maillefer, Konstanz, Germany.
Epiphany	BisGMA, ethoxylated BisGMA, UDMA, hydrophilicdifunctional methacrylates.	Calcium hydroxide, barium sulphate, barium glass, bismuth oxychloride, silica.	Epiphany SE Pentron Clinical Technologies, Wallingford, CT, USA.
GuttaFlow	(i) Polydimethylsiloxane particles. (ii) Silicone. (iii) Paraffin oil. (iv) Platinum catalyst. (v) Zirconium dioxide. (vi) Nano-silver. (vii) Gutta-percha powder.		GuttaFlow Coltene-whaledent Langenau, Germany.

and medicaments may leach through dentin [10] or when extruded beyond the root apex and cause a cytotoxic damage [11]. Nonresorbable sealers may cause a cytotoxic effect for several days due to polymerization derivatives release such as formaldehyde [12]. Regardless, these sealers are widely used in the clinical daily practice and are accepted owing to their chemical stability.

Based on the above, the antimicrobial activity of endodontic sealers may be an important tool for infection control. To reach this goal, antibacterial quaternary ammonium polyethylenimine (QPEI) nanoparticles were incorporated into conventional endodontic sealers. Previously, we showed that when these nanoparticles were immobilized into resin-based materials, they caused a strong, long-lasting antibacterial effect upon contact without leaching of the nanoparticles and without compromise of the mechanical properties [13]. Furthermore, dental restorative composites incorporating up to 2% wt/wt QPEI nanoparticles caused no inflammatory *in vivo* response [14].

It seems that incorporation of QPEI nanoparticles into endodontic sealers may be beneficial in achieving antibacterial activity [13]. Nonetheless, biocompatibility of modified endodontic sealers with antibacterial nanoparticles needs to be established by comparing these new materials to nonmodified sealers. In the present study, the cytotoxic effect was tested using fibroblasts and macrophages cells. These cells have been shown to play a critical role in the biological response to materials [15]. Due to the fact that macrophages direct much of the chronic inflammatory response, the ability of a material to alter a cell's viability or secretion function may have significant consequences on the overall biological response to a given material [16].

The aim of the present study was to compare the cytotoxic effect of 3 commercially available sealers prior and following incorporation of 1%, or 2% QPEI on macrophages and fibroblast cell lines and on the secretion of TNF $\alpha$  from the macrophages.

## 2. Materials and Methods

**2.1. QPEI Nanoparticle Preparation.** The synthesis of quaternary ammonium PEI nanoparticle was previously described by Beyth et al. [13]. Briefly, PEI (10 g, 0.23 mol monomer units) dissolved in 100 mL ethanol was reacted with dibromopentane at a 1:0.04 mol ratio (monomer units of PEI/dibromopentane) under reflux for 24 hrs. N-alkylation was conducted as follows: octyl halide was added at a 1:1 mol ratio (monomer units PEI/octyl halide). Alkylation was carried out under reflux for 24 hrs, followed by neutralization with sodium hydroxide (1.25 equimolar, 0.065 mol) for an additional 24 hrs under the same conditions. N-methylation was conducted as follows: 43 mL (0.68 mol) of methyl iodide were added and methylation was continued at 42°C for 48 hrs followed by neutralization with sodium bicarbonate (0.23 mol, 19 g) for an additional 24 hrs. The supernatant obtained was decanted and precipitated in 300 mL of double distilled water (DDW), washed with hexane and DDW, and then freeze-dried. The purification step was repeated using additional amounts of hexane and DDW. The average yield was 70% (mol/mol).

**2.2. Preparation of the Test Samples.** The tested materials AHPlus, Epiphany, and GuttaFlow (Table 1) were prepared according to the manufacture instructions and applied on

TABLE 2: Summary of atomic percentage values of elements determined by XPS analysis and DLS measurements.

Tested material	Atomic concentration % / Mass concentration %				DLS Analysis
	I 3d	O 1s	C 1s	Si 2p	
AH Plus	—	21.79/21.85	59.08/44.48	19.13/33.67	—
AH Plus + 2% QPEI	0.08/0.64	21.17/21.46	61.09/46.48	17.66/31.42	—

AHPlus with and without added QPEI nanoparticles.

<sup>a</sup>QPEI nanoparticle presence in the modified sealers was estimated using X-ray photoelectron spectroscopy; using Kratos Axis Ultra spectrometer (Kratos Analytical Ltd., Manchester, UK), using Al K $\alpha$  monochromatic radiation X-ray source (1,486.7 eV). AH plus discs incorporating 0% or 2% wt/wt were prepared and left to dry in the incubator for 1 week.

<sup>b</sup>Supernatant of AHPlus with and without QPEI nanoparticles was determined using dynamic light scattering analyzer (Zetasizer Nano-S, Malvern); samples ( $n = 8$ ) were incubated for 1 week; supernatant was collected and tested—no particles.

specially designed plastic inserts. The nanoparticle powder was added at 0, 1, or 2% wt/wt to each of the endodontic sealers and homogeneously mixed according to the manufacturer's instructions. These plastic inserts were sterilized under gas condition for 24 hrs and placed in a 96-well tissue culture plate directly to the cells. In some of the experiments, the inserts were immersed in media for another 24 hrs, for residual materials washing, before challenging the cells.

**2.3. Material Characterization.** The FT-IR spectra of the QPEI nanoparticles were recorded on a Perkin-Elmer, 2000 FTIR. FT-IR: 3,440  $\text{cm}^{-1}$  (N-H), 2,956, 2,926, and 2,853  $\text{cm}^{-1}$  (C-H), 1,617  $\text{cm}^{-1}$  (N-H, small band), 1,465  $\text{cm}^{-1}$  (C-H), 967  $\text{cm}^{-1}$  quaternary nitrogen. <sup>1</sup>H-NMR (DMSO): 0.845 ppm (t, 3H, CH<sub>3</sub>, octane hydrogens), 1.24 ppm (m, 10H, -CH<sub>2</sub>-, octyl hydrogens) 1.65 ppm (m, 2H, CH, octyl hydrogens), 3.2–3.6 ppm (m, CH<sub>3</sub> of quaternary amine, 4H, -CH<sub>2</sub>-, PEI hydrogens and 2H, -CH<sub>2</sub>-, octyl hydrogens. QPEI nanoparticle presence in the modified sealers was estimated using X-ray photoelectron spectroscopy (XPS). X-Ray photoelectron spectra (XPS) were recorded using Kratos Axis Ultra spectrometer (Kratos Analytical Ltd., Manchester, UK), using Al K $\alpha$  monochromatic radiation X-ray source (1,486.7 eV). The emission current was set for 15 mA, and the anode high voltage to 15 kV. All XPS spectra were collected with 90° take off angle (normal to analyzer), vacuum condition in the chamber was 1.9·10<sup>-9</sup> Torr. The survey XPS spectra were acquired with pass energy 160 eV and 1 eV step size, and the high-resolution spectra were collected for C 1s, O 1s, Si 2p, Zr 3d and I 3d levels, with pass energy 20 eV and 0.1 eV step size. Additionally, the leaching of QPEI nanoparticles from the modified sealers was recorded using DLS measurements. Samples' size distributions were evaluated by a dynamic light scattering analyzer (Zetasizer Nano-S, Malvern. Surface characterization of the nonmodified versus the modified sealers is summarized in Table 2. Nanoparticle electronic microscopy and full characterization of the QPEI nanoparticles was previously described by Yudovin-Farber et al. [17].

**2.4. Cell Cultures (Macrophages and Fibroblast Cells).** The raw 264.7 macrophage cell line and fibroblast (CCL-1 L-929 american type culture collection) cell lines were cultured separately in Petri dishes in Dulbecco's minimum

essential Medium (DMEM) supplemented with 10% fetal calf serum (FCS), 1% penicillin/streptomycin, and 1% glutamine. Before the assay, the cells were seeded at a density of 60,000 cells in 200  $\mu\text{L}$  media per well in 96-well tissue culture plates (NUNC). Twenty-four hours after plating, the cells were activated by 10  $\mu\text{L}$  of heat-killed *Porphyromonas gingivalis* 33277 ATCC and exposed to the materials layered on the special inserts that was placed in the wells. Following 24 hrs of incubation at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>, the plates were analyzed for cell viability and the secreted levels of TNF $\alpha$  of macrophages. Based on our preliminary data on the kinetics of TNF $\alpha$  secretion, the cytokine level was tested by ELISA following 24 hours of *P. gingivalis* challenge.

**2.5. Cell Viability.** The viability of the cells was evaluated using a colorimetric XTT assay as described by Scudiero et al. [18]. At the end of incubation the suspended cells were exposed to the tested materials, the cells were centrifuged and all cells were subjected to XTT assay. Results present the total effect of the materials on the cells in all groups compared to control. The assay is based on the ability of metabolically active cells to reduce the tetrazolium salt XTT to orange-colored compounds of formazan. Following 24 hrs of incubation, 50 mL of XTT labeling mixture were added to each well and the microplate was incubated for a further 4 hrs. A Vmax microplate reader (Molecular Devices, Palo Alto, CA) with a 450 nm optical filter and a 650 nm reference wavelength was used to measure the absorbance of each well.

**2.6. Cytokine Analysis.** The presence of cytokines was determined by two-site ELISA [19]. The TNF $\alpha$  assay was based on commercially available antibody pairs (Pharmingen, San Diego, CA). The 96-well ELISA plates were coated with 1 mg/mL anti-mouse cytokine monoclonal antibodies, and blocked with 3% bovine serum albumin (BSA). After addition of the samples, a secondary biotinylated antibody was used as the detecting antibody, followed by a streptavidin-horseradish peroxidase conjugate (Jackson Immunoresearch Laboratories, West Grove, PA). The substrate used was o-phenylenediamine (Zymed, San Francisco, CA). The reaction was terminated by the addition of 4 N sulfuric acid, and the optical density was read with the aid of a Vmax microplate reader (Molecular Devices) at 490–650 nm.

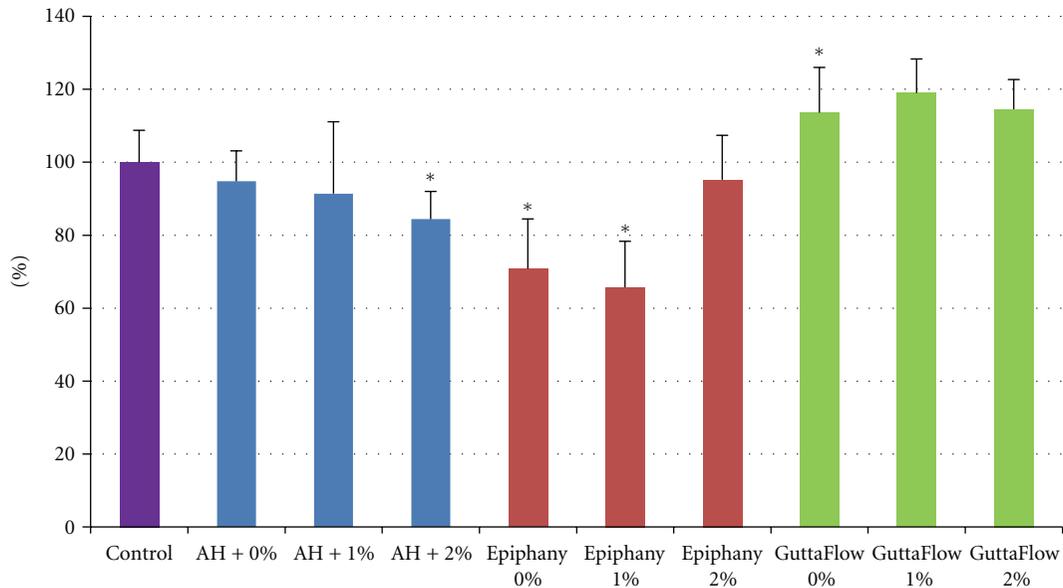


FIGURE 1: The effect of AH+, Epiphany, and GuttaFlow endodontic sealers on the mitochondrial activity of the L-929 fibroblast cell line. Mitochondrial activity was normalized to the control without sealers stimulation. Stimulated fibroblasts (with heat-killed *P. gingivalis*) with and without the various endodontic sealers were cultured for 24 hrs. Cell viability was tested using the XTT test. The results are expressed as the mean + SD. Statistically significant differences between the control (media) and the different groups are indicated by asterisks ( $P < 0.05$ ) and  $n = 8$  wells.

**2.7. Heat-killed Bacterium *P. gingivalis*.** *P. gingivalis*, strain ATCC 33277, was grown on blood agar plates in an anaerobic chamber with 85% N<sub>2</sub>, 5% H<sub>2</sub>, 10% CO<sub>2</sub>. After incubation at 37°C for 2-3 days the bacteria were inoculated into peptone yeast extract and incubated for 3-4 days, under the same conditions. To obtain heat-killed bacteria, the bacteria were washed 3 times with PBS and then exposed to 80°C for 10 min [20]. The bacterial concentration was standardized to an optical density of OD<sub>650</sub> = 0.1, corresponding to 10<sup>10</sup> CFU/mL [21]. The heat-killed bacteria were stored at 4°C until used when they were resuspended in solution by brief sonication.

**2.8. Statistical Analysis.** Data analysis was performed using SigmaStat statistical software (Jandel Scientific, San Rafael, CA). One way repeated measure of analysis of variance (RM ANOVA) was used to test the significance of the differences between the treated groups. If significance was established, the intergroup differences were tested for significance using Student's *t*-test with the Bonferroni correction for multiple testing. The significance of the differences between two treatment groups was evaluated using the *t*-test. The level of significance was set at  $P < 0.05$ . All the results are presented as the mean ± standard error.

### 3. Results

**3.1. The Cytotoxic Effect on Fibroblasts.** The cytotoxicity of endodontic sealers incorporating 0, 1, or 2% wt/wt was evaluated using the XTT test. Fibroblasts were exposed to the tested materials for 24 hrs, and their viability was

normalized according to the control (cells without any material) mitochondrial activity and evaluated. Results show that the fibroblast's viability varied when exposed to the different sealers: in the presence of AHPlus viability of the cells was not affected when compared to the control, Epiphany caused significant decrease ~20% in the cell viability and GuttaFlow increased cell viability. The addition of antibacterial nanoparticles had an insignificant effect on cell viability when compared to the nonmodified endodontic sealer material: cell viability was similar in the presence of Epiphany and GuttaFlow with or without added QPEI nanoparticles, AHPlus incorporating 2% QPEI nanoparticles caused some decrease in cell viability only no significant decrease was seen in the 1% group (Figure 1).

Immersion of the test materials after their setting in media for 24 hrs, did not affect the viability of the fibroblasts. The same pattern of cytotoxicity of the different sealers independent of nanoparticles incorporation can be seen in Figure 2. AHPlus sealer incorporating 2% QPEI was the only group to show a slight decrease in fibroblast viability compared to the nonmodified sealer.

**3.2. The Cytotoxic Effect on Macrophages.** Macrophages were exposed to the tested materials for 24 hrs, and their viability was normalized according to the control (cells without any material) mitochondrial activity. Two of the three different sealers, AH Plus and Epiphany, caused a decrease in macrophage viability, while GuttaFlow had no effect when compared to control. The incorporation of the nanoparticles into the sealers had no effect when added at 1% wt/wt, whereas when 2% wt/wt of nanoparticles were incorporated

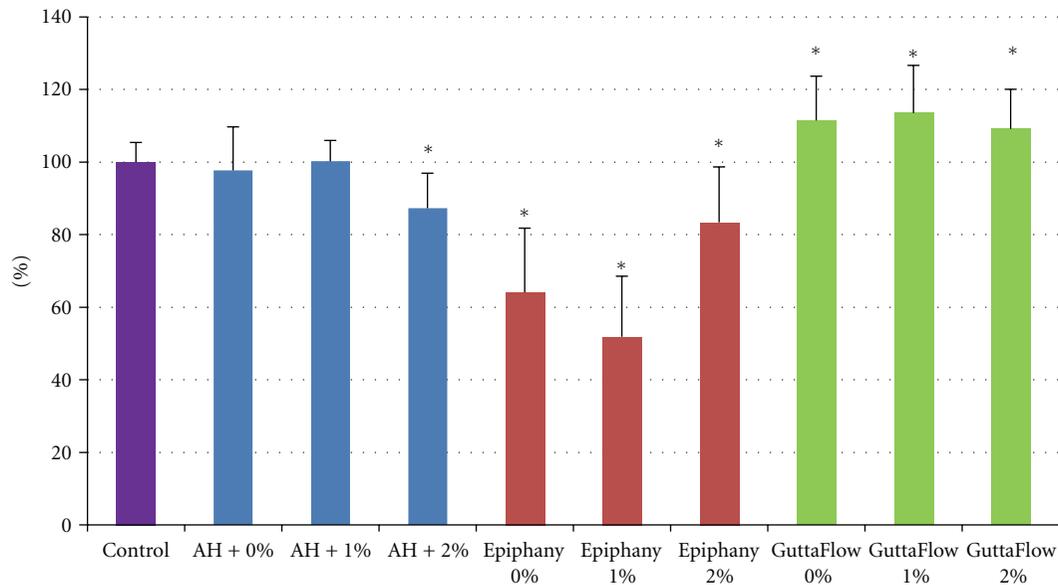


FIGURE 2: The effect of AH+, Epiphany and GuttaFlow endodontic sealers on the mitochondrial activity of the L-929 fibroblast cell line after 24 hrs immersion in media. Mitochondrial activity was normalized to the control without sealers stimulation. Stimulated fibroblasts (with heat-killed *P. gingivalis*) with and without the various endodontic sealers were immersed in media for 24 hrs, and then transferred to cell cultured fibroblasts for another 24 hrs. Cell viability was tested using the XTT test. The results are expressed as the mean + SD. Statistically significant differences between the control (media) and the different groups are indicated by asterisks ( $P < 0.05$ ) and  $n = 8$  wells.

reduced viability of the cells was seen in all three sealers when modified (Figure 3).

Immersion of the test materials after their setting on the inserts in media for 24 hrs, reduced the cytotoxicity of all three materials. The viability of the macrophages was similar to the control (Figure 4). Incorporation of the nanoparticles into the sealers had no effect when added at 1% wt/wt, whereas when 2% wt/wt of nanoparticles were incorporated reduced viability of the cells was seen with all the tested materials similarly as in the nonwashed materials (Figure 4).

**3.3. TNF $\alpha$  Secretion by Macrophages.** Macrophages were exposed to the materials for 24 hrs combined with heat-killed *P. gingivalis* challenge to stimulate the cells. The secretion of TNF $\alpha$  by macrophages was measured and normalized according to the control (cells with bacterial challenge alone). Challenged macrophages showed reduced secretion of TNF $\alpha$  in the presence of AHPlus and Epiphany. Similar TNF $\alpha$  secretion was seen when nanoparticles were incorporated into AH Plus with no effect on macrophage activity. However, incorporation of nanoparticles into Epiphany reduced the levels of TNF $\alpha$  to nondetectable (Figure 5). The effect of GuttaFlow on macrophage activity seemed neglectable. Both the nonmodified sealer and the sealer incorporating 1% or 2% nanoparticles did not affect TNF $\alpha$  secretion and the levels were similar to the control with no statistical significance.

#### 4. Discussion

Results show that the incorporation of 1% wt/wt of QPEI nanoparticles into endodontic sealers has no cytotoxic effect

for all three tested materials. The viability of both cell lines: fibroblasts and the macrophages remain stable when compared to the nonmodified sealers. The incorporation of 2% nanoparticles seems slightly toxic depending on: the tested material, the culture conditions, and the cell type. This diverse effect of the materials with different cell lines is a very important outcome for future designing cytotoxic studies.

Endodontic sealers are routinely used to fill gaps within the solid components of the root canal filling and allow its adaptation to the dentin walls [4]. Since root canal filling is the ultimate barrier between the oral cavity and inner body (e.g., periodontal ligament and bone) an antibacterial effect is desirable. However, previous attempts to render sealers with antibacterial properties resulted in severe cytotoxic effect [22]. This effect was evident when sealer leaching through dentin various ports of exit from the root canal system to the tooth surrounding tissues occurred [10, 11, 14]. Such events were reported to be responsible for cases of pain, nerve paresthesia, anesthesia and delayed healing due to their cytotoxic effect. More troubling is the possible cytotoxic effect to remote organs once these materials are dissolved [23].

Several materials constituting endodontic sealers were recognized as cytotoxic. For example, eugenol that was proved to leach out of zinc-eugenol based sealers was found cytotoxic to nerve cells as well as to human periodontal ligament (PDL) fibroblasts [24–26]. Another example is paraformaldehyde that was vastly used in the past and is a byproduct of contemporary epoxy resin based sealers [27]. Paraformaldehyde was reported to be cytotoxic and mutagenic [28]. Another component that is released during setting is polyketone. It can be found in the polyketone-based cement Diaket which was reported to be cytotoxic to

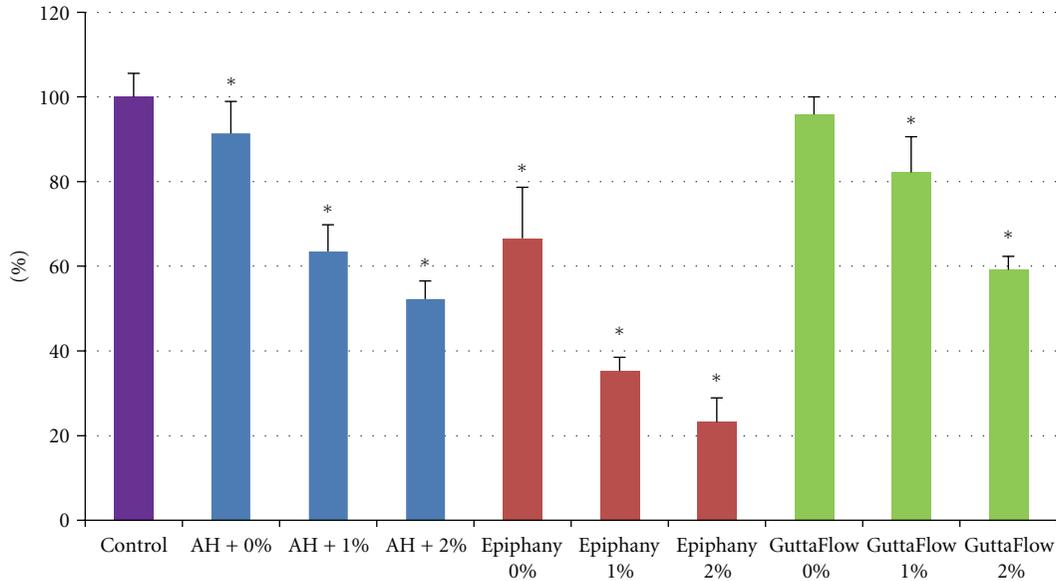


FIGURE 3: The effect of AH+, Epiphany and GuttaFlow endodontic sealers on the mitochondrial activity of the RAW macrophage cell line. Mitochondrial activity was normalized to the control without sealers stimulation. Stimulated macrophages (with heat killed *P. gingivalis*) with and without the various endodontic sealers were cultured for 24 hrs. Cell viability was tested using the XTT test. The results are expressed as the mean + SD. Statistically significant differences between the control (media) and the different groups are indicated by asterisks ( $P < 0.05$ )  $n = 8$  wells.

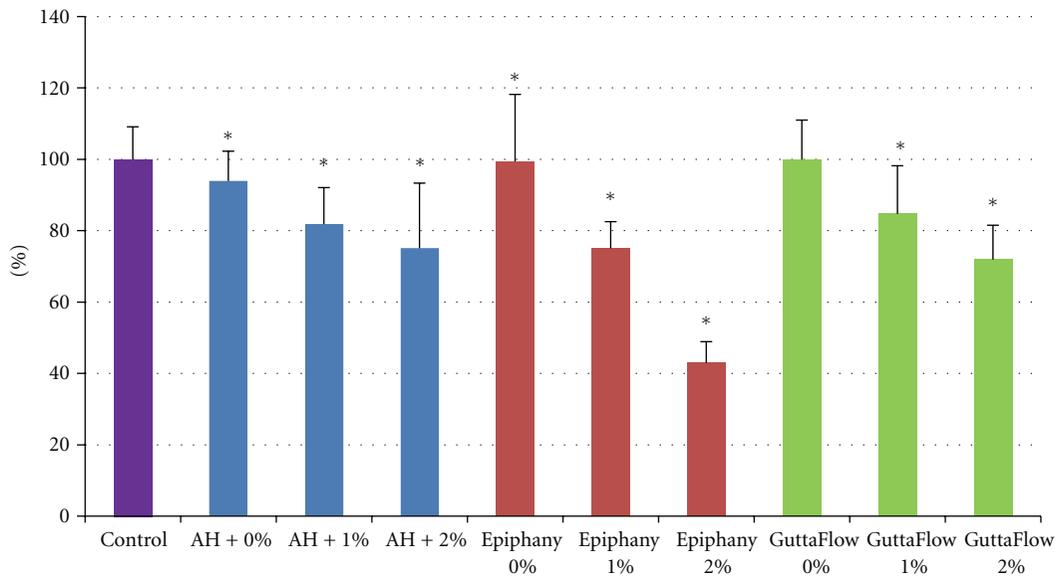


FIGURE 4: The effect of AH+, Epiphany, and GuttaFlow endodontic sealers on the mitochondrial activity of the RAW macrophage cell line after 24 hrs immersion in media. Mitochondrial activity was normalized to the control without sealers stimulation. Stimulated macrophages (with heat-killed *P. gingivalis*) with and without the various endodontic sealers were immersed in media for 24 hrs and then transferred to cell cultured macrophages for another 24 hrs. Cell viability was tested using the XTT test. The results are expressed as the mean + SD. Statistically significant differences between the control (media) and the different groups are indicated by asterisks ( $P < 0.05$ ) and  $n = 8$  wells.

HeLa cells and L-645 fibroblast cells [29]. Another group of materials that was reported to release cytotoxic components such as triethylene glycol dimethacrylate monomer, urethane dimethacrylate (UDMA), HEMA initiators, and silica are resin-reinforced materials [19].

In the present study, it was observed that the cytotoxic effect differed between materials. AHPlus caused a moderate cytotoxic effect that could be attributed to bisphenol A diglycidyl ether release. This resin-based component is known to be potentially cytotoxic and mutagenic [30]. In addition,

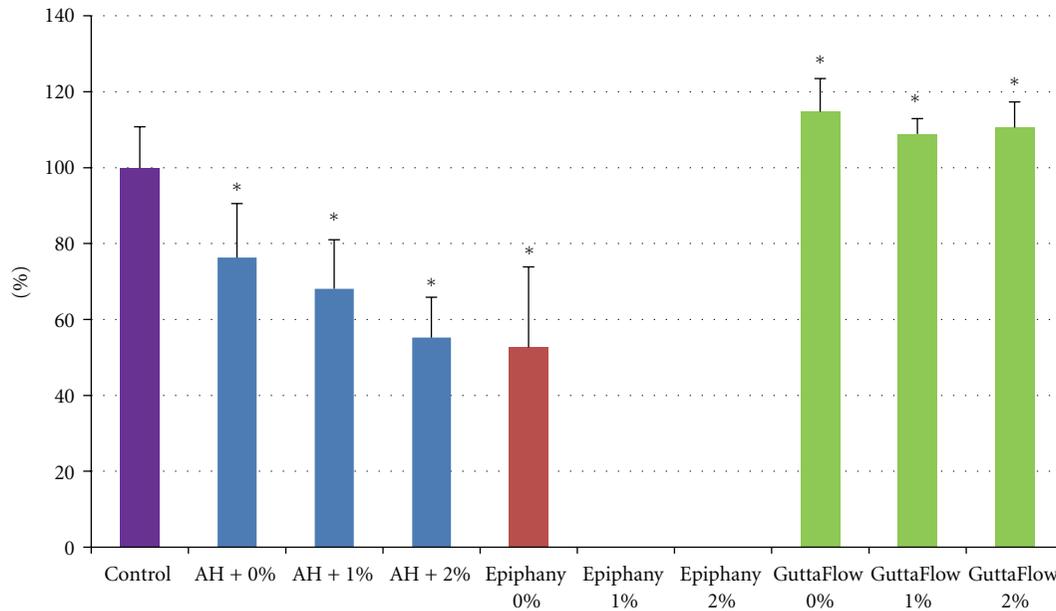


FIGURE 5: The effect of AH+, Epiphany, and GuttaFlow endodontic sealers on TNF $\alpha$  secretion in the RAW macrophage cell line. Mitochondrial activity was normalized to the control without sealers stimulation. Stimulated macrophages (with heat-killed *P. gingivalis*) with and without the various endodontic sealers were cultured for 24 hrs in the presence of heat-killed *P. gingivalis*. The secreted TNF $\alpha$  levels in the media were analyzed by ELISA. The results are expressed as the mean + SD. Statistically significant differences between the control (media) and the different groups are indicated by asterisks ( $P < 0.05$ ) and  $n = 8$  wells.

the cytotoxic effect can be attributed to the release of small amounts of formaldehyde or amine and epoxy resin components from the sealer [31]. The resin component urethane dimethacrylate (UDMA) that can be found in EndoRez sealer was reported to cause intracellular glutathione depletion even at low concentrations within a short period of time resulting in a cytotoxic effect [32]. Resin-based endodontic sealers such as Epiphany or Metaseal consist of polymerized resin components reinforced by inorganic fillers. The resin matrix comprise a mixture of bisphenol A-glycidyl methacrylate (Bis-GMA), ethoxylated Bis-GMA, UDMA, and hydrophilic difunctional methacrylates [33], all of which when leaching out may produce a toxic effect. Metaseal contains HEMA which is reported to inhibit intracellular tyrosine phosphorylation in L929 cells [34]. It also induces cell-growth inhibition and cycle perturbation as well as glutathione depletion and reactive oxygen species production [35]. Glutathione depletion was reported to be responsible for the cytotoxic effect of eugenol [36]. Formaldehyde releasing sealers such as AH26 and N2, were shown to downregulate alkaline phosphatase causing inhibition of new bone formation [37].

*In vitro* cytotoxicity evaluation of endodontic sealers provides a controlled setup that allows quick evaluation and comparison between different sealers. Previous studies showed that a silicon-based sealer (Roekoseal) and an epoxy resin-based sealer (AHPlus) are non-toxic when freshly prepared or following a setting period of one to seven days, showing 40–60% cells viability as compared to Teflon controls. Under the same conditions Epiphany was found to

be highly cytotoxic showing 0% viability [38]. Similarly, 12-week-aged AHPlus retained its ability to suppress cytokine secretion of monocytic cells without induction of secretion of TNF $\alpha$ , IL $\beta$ , or IL6. One year analysis of elutes of AH Plus and Roekoseal showed minimal cytotoxic effect on 3t3 fibroblasts as well as on periodontal ligament fibroblasts [9]. A study by Al-Hiyasat et al. showed that different cytotoxic effect of AHPlus and Epiphany depends on magnitude of dilution of the samples [19]. Contradictory to traditional root canal sealers that polymerize inside the root canal and may release antibacterial and cytotoxic derivatives as byproducts, QPEI are prepolymerized and do not release any by products. QPEI was reported to possess an excellent antibacterial activity [39]. When these polymers are synthesized as insoluble antibacterial nano-sized particles and are incorporated into resin base materials, potent and long-lasting antibacterial surface properties can be attained *in vitro* and *in vivo* [13, 40]. Usage of nanomaterials altering surface properties has been shown suitable for various biomedical applications [41, 42]. QPEI is a stable antibacterial compound that does not leach out from the material into the surrounding environment rendering perpetual antibacterial surface properties. The present study results coincide with our previous results showing that the incorporation of QPEI nanoparticles does not affect the base material's biocompatibility.

Incorporation of QPEI nanoparticles into noncytotoxic endodontic sealers such as silicon-based sealers may provide added value of antibacterial properties without compromising the materials' biocompatibility. Furthermore, this

antibacterial effect is expected to last longer as QPEI nanoparticles are nonleachable components encapsulated in the material matrix while the antibacterial effect of traditional sealer is self-limited to the setting or degradation periods.

### Conflict of Interests

A patent entitled “Antimicrobial Nanoparticulate Additives Forming Non-Leachable Sustained Antimicrobial Polymeric Compositions” is pending approval.

### Author’s Contribution

I. Aramovitz and N. Beyth contributed equally to this work.

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