

Toxicity of endodontic filling materials

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The toxicity of endodontic materials is reviewed. The patient may be exposed by constituents leaching from the unset and set sealer and by accidental overfilling of the root canal. For most patients the exposure is believed to be below the threshold for a toxic effect. Most sealers exhibited a cytotoxic response, especially unset and the responses diminished with time after setting. Unset and newly set endodontic material may also have a toxic potential *in vivo* that may result in a localized inflammation postponing the healing of an apical periodontitis. Root canal sealers containing paraformaldehyde is of biological concern.

This paper deals with the toxic reactions elicited by endodontic filling materials. Such reactions may occur in any patient, provided the exposure exceeds a certain level. The threshold level is based on the fact that the human body has the ability to detoxify xenobiotics (= compound foreign to the body), but when the exposure exceeds this ability, a toxic response occurs. A toxic reaction is also dose-related in that the biological response increases by increasing dose.

The toxic response may be investigated on several levels, i.e. using cells and tissue from animals, animal studies and observations from clinical investigations addressing product suitability. Clinical observations are most relevant, but have the disadvantage that unknown or uncontrolled factors may influence the results. Such factors are more easily controlled in animal studies and in *in vitro* experiments; on the other hand, such studies may be less relevant for the clinical situation.

Exposure

The filling of an endodontically instrumented and disinfected tooth is based on two concepts, sealer cements used with a core and filler material used without core. Types of materials used for endodontic filling procedures are listed in Table 1. Following a normal endodontic filling procedure, the contact area between the patient and the filling material is limited to the apical opening of the root canal(s). As the setting reaction of the sealer and the filling material takes place

in the tooth, leaking of constituents from the unset material into the periapical tissue is possible. Another possibility of unwanted exposure occurs when the canal is overfilled. In cases of overfilling the canal, the material comes into direct contact with the periapical tissues and may also affect neighbouring anatomical structures such as the mandibular canal or the maxillary sinus. Products leaching from the material over time through accessory canals and dentinal tubules expose the tissue surrounding the tooth. Mostly, the exposure from endodontic filling materials is believed to be low and below the threshold for what is considered a toxicologic response.

In vitro toxicity

Cell culture studies are usually the starting point of a biocompatibility evaluation. Cell culture studies provide toxicological information in a simplified system that minimizes the effect of confounding variables. Various primary cells, cell lines and biological endpoints have been used for the evaluation of cytocompatibility of endodontic filling materials, and there is still a controversy over what is the most relevant methodology (1).

Core materials

Gutta-percha has been evaluated in various cell culture systems, and found to elicit no or low cytotoxicity (2). It was found that cytotoxic effects varied among brands.

Table 1. Endodontic filling materials

Function	Material	Main composition
Core materials	Gutta-percha	Gutta-percha, zinc oxide, metals, colophonium, pigments
	Resin coated gutta-percha	Resin (not specified), gutta-percha, zinc oxide
	Thermoplastic polymer	Polyester, bioactive glass, bismuth oxychloride, barium sulphate
	Titanium	Titanium
Sealers	Zinc oxide eugenol	Zinc oxide, eugenol, bismuth, barium
	Polyketone	Zinc oxide, propionylacetophenone, vinyl copolymer
	Epoxy	Bisphenol-A diglycidylether, bismuth oxide/zircon oxide
	Calcium hydroxide	Ca(OH) ₂ , colophonium, different oxides, salicylate-based activator
	Silicone	Addition silicone
	Resin Based	Bis-GMA, UDMA, hydrophilic difunctional methacrylate, CaOH, Barium Sulphate, Barium Glass
	Glass ionomer	Fluoroaluminosilicate glass, polyacrylic acid
	Resin modified glass ionomer	Fluoroaluminosilicate glass, polyacrylic acid, water-soluble methacrylate monomer (HEMA)

Data from resin-coated gutta-percha is lacking. Resin-based materials cured *in situ* have the potential of eliciting cytotoxic responses (3). However, the curing of the resin-coated gutta-percha cones are probably better than that obtained for resin materials *in situ*, making a biological response to resin-coated gutta-percha cones less likely to occur. Preliminary results from biocompatibility studies with thermoplastic polymer core material in our laboratory indicated a cytotoxic response. When titanium is used as core material, the surface is covered with a passivating titanium oxide film. Cell culture studies addressing various endpoints like proliferation, differentiation and protein syntheses have revealed no negative biological response to titanium (4–7).

Sealers

Eugenol is found to leak from zinc oxide-eugenol sealers (8). Both zinc oxide-eugenol and eugenol induce a toxic effect in cell culture models (9, 10), and reduce the transmission in nerve cells (11). The effects are persistent, also after setting of the material. Zinc oxide-eugenol sealer with paraformaldehyde is both cytotoxic and mutagenic (12, 13). Polyketone

sealers also exhibited a cytotoxic effect, which diminished after complete setting of the material (10, 14). Inconsistent results have been obtained in cell culture studies with epoxy-based sealers, partly related to variation in time interval between mixing of the material and start of testing in the various studies (2). In an earlier literature survey, epoxy-based materials were deemed as highly cytotoxic. Formaldehyde is released from some epoxy-based materials during and shortly after setting (15, 16). This fact and the results from cell culture studies indicate that epoxy-based materials have a high initial cytotoxicity that diminishes by aging of the test specimens. Several authors report good cytocompatibility of sealers based on calcium hydroxide (for a review, see (1)). The initial high pH from calcium hydroxide in these products had to be buffered by the cell culture medium to obtain the favorable results (2). Data on silicone- and resin-based sealers are scarce. Preliminary data from our laboratory indicate low cytotoxicity for silicone sealers and comparably higher cytotoxicity for resin-based sealers. Cell culture studies with glass ionomers showed high cytotoxic response for freshly mixed test specimens, and low to no cytotoxicity when the material was set (17). Both resin-reinforced and

conventional glass ionomers that are exposed to water before the setting reaction is completed release high amounts of aluminum (18). In aged test specimens the release of aluminum is low, and the difference in aluminum release between newly set and aged test specimens has been suggested as an explanation for the difference in cytotoxicity (19). For resin-reinforced materials, leaching out of triethylene glycol dimethacrylate monomer and initiators may contribute to the cytotoxicity (20).

Genotoxicity

For assessment of the genotoxic potential of any material, it is recommended to perform a series of *in vitro* tests. At least two assays, investigating different endpoints, shall use mammalian cells (21). For cytotoxic and bactericidal compounds, as many endodontic sealers appear to be (1), care must be taken in the test set up: For a proper evaluation the selected test concentrations used for genotoxic effects must be below the concentrations where toxic effects are found.

Paraformaldehyde-containing zinc oxide-eugenol- and epoxy-based sealers are mutagenic in tests using bacteria (12, 13). Epoxy-based sealers are also mutagenic in mammalian cell mutation assays (22). The mutagenic effects disappear shortly after setting of the material. Formaldehyde is released from some epoxy-based sealers with a maximum after 2 days, even though the amount is much less than that of paraformaldehyde-containing zinc oxide-eugenol sealers (15). It was believed that the leakage of formaldehyde and bisphenol-A diglycidyl ether from the epoxy-sealers contributed to the mutagenic effects (13, 22).

Both formaldehyde and bisphenol-A diglycidyl ether have been evaluated by the International Agency for Research on Cancer under WHO. Formaldehyde is classified as a carcinogen in animals, whereas there exists only limited evidence for carcinogenic effects in man (23). There is also limited evidence for animal carcinogenicity from bisphenol-A diglycidyl ether and no adequate data for the evaluation of human cancer risk for this compound (24). Considering the limited exposure of these compounds from endodontic epoxy-sealers and the lack of definitive assessment by the IARC, it seems unlikely that such sealers contribute to an increased risk of cancer in patients. However, the high level of paraformaldehyde in zinc oxide-eugenol

sealers containing paraformaldehyde may be a matter of possible concern (1).

In vivo toxicity

Core materials

Toxicity testing of gutta-percha products has revealed inconsistent results (for a review, see (1–2)). Implantation studies in both soft and hard tissues resulted in reactions varying from none to chronic inflammation depending on the gutta-percha product used. Variation in the composition of the gutta-percha cones is a likely explanation, even though a detailed description of the composition was lacking in most studies. The inflammation observed in these studies may have been induced by complement activation associated with the gutta-percha compound, antioxidants and oxides (25). Systemic reactions to gutta-percha products have not been reported (2). A number of animal studies have documented excellent biocompatibility of titanium (26). A thin surface layer of titanium oxide that is highly resistant to corrosion is the most important factor for its favorable biological properties (27). For other core materials, *in vivo* data are lacking.

Sealers

Implantation of zinc oxide-eugenol sealers have resulted in localized inflammation both in soft tissue observed up to 120 days (28, 29) and in bone up to 3 months (30). In soft tissue, the intensity of the reaction diminished by the 60th day, and this reduction continued progressively through the 120th day (29). The effects of zinc oxide-eugenol-based root canal sealers on an experimentally-induced apical periodontitis were studied histologically in rats. Root canal sealers containing paraformaldehyde essentially impaired periapical repair (31).

Both sub-cutaneous and intra-muscular implantation of polyketone-based sealers have resulted in local tissue reaction that diminished by increasing observation time (32, 33). Epoxy-based sealers have been implanted in different tissues (skin, muscle and bone) of laboratory animals and the effect evaluated by histopathology. A general finding was initial inflammation that subsequently diminished when the observation time was

prolonged (2, 33). For example, no response was seen 3 months after implantation in bone. Also calcium hydroxide-based sealer gave an initial inflammatory response when implanted subcutaneously (29). The effect ceased by the end of the 3-month observation period, and the reaction of the calcium hydroxide-based sealer was deemed to be somewhat more favorable than that of zinc oxide eugenol in a short-term perspective (29). For the other sealer materials, addition-type silicone and resin-based materials, *in vivo* data apparently has not yet been published.

Sub-cutaneous implantation of glass ionomer sealer in rats resulted in an initial inflammation that could not be observed after 3 months (34). Resin modified glass ionomer cement sealer has also been used as pulp capping material with results suggesting acceptable biocompatibility (35). Glass ionomer cements are well tolerated by bone tissue and have been considered suitable as retrograde root filling material (36, 37), although tests in dogs have shown less favorable results with this type of materials (38).

Concluding remarks

Systemic toxic reactions induced by endodontic materials have not been reported (2). This observation is not unexpected as the exposure from root canal filling materials must be regarded as low and probably below the level necessary to induce a systemic effect. However, both cell culture studies and implantation studies in animals have revealed a toxic potential of especially unset and newly set endodontic material. This may result in a localized inflammation and influence the healing of an apical periodontitis.

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