# Summary

Materials used for root canal filling should permanent obturate the root canal space after it has been chemo-mechanical prepared. Despite sealing ability of root canal filing also biocompatibility is affecting the success of root canal treatment.

Due to a long-term contact of obturation materials with the periapical tissues, their toxic effect can damage tissues or hinder healing of inflamed periapical structures. Recently, many new materials have been introduced into dental market and there are conflicting reports, in the literature, regarding their biocompatibility.

The aim of the study were:

1. Compare the cytotoxic effects of recently used root canal filing materials, immediately after mixing and after setting
2. Attempt to explain a role of oxidative stress in promoting cytotoxicity of root canal obturation materials
3. Assessment of cellular death type, following the exposure to tested materials.

In the study human periodontal ligament fibroblasts (Cell System HPdLFCloneticsTM, Lonza Walkersville, Inc., Walkersville, USA) were used.

Cells routinely cultivated in DMEM (Dulbecco’s Modified Eagle’s Medium; Merk Life Science, Darmstadt, Germany) supplemented with 10% fetal bovine serum (FBS) (Merk Life Science, Darmstadt, Germany), 100 µg/mL penicillin, and 100 µg/mL streptomycin at 37°C, 5% CO2, and 95% humidity. The experiment was performed using the root canal filling materials- gutta -percha pellets (GP), resilon (RLN) and endodontic sealers: MTA Fillapex (FL), RealSeal SE (RSEAL), MetaSeal Soft (META), AH Plus (AH), Roeko Seal Automix (RSA), GuttaFlow (GF), Apexit Plus (AP), Sealapex (SP), Endomethasone N (EN), Tubliseal (TS). Immediately after preparation, materials were applied into a plastic rings. I group were materials immediate after mixing; II group- materials after setting which were stored for 24 hours at 37°C, 5% CO2, and 95% humidity. Rings with materials were placed into inserts, transferred into tissue culture plates with HPdLFs. Fibroblasts were incubated with materials (“fresh” and “set”) for 24 hours. Gutta-percha pellets and resilon were assessed as a set samples. Assessment the toxic potential of the materials was performed using an assay examining mitochondrial enzyme activity   
3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay). Measuring intracellular reactive oxygen species (ROS) was performed   
by 2',7'-dichlorodihydrofluorescein diacetate using flow cytometry. Apoptosis and necrosis quantitative cell assessment was investigated with Annexin V-FITC Apoptosis Kit using flow cytometry. The results were subjected to statistical analysis using the SPSS 21.0 package. The signiﬁcance level was p<0.05.

During cytotoxicity assessment with MTT test, Dahl et al. criteria were applied: non-cytotoxic – cell viability according to control group > 90%; mild- 60 % - 90 %; moderate 30 % – 59 %; severe< 30 %. None of the group I materials, demonstrated severe cytotoxicity. META (34.36% ± 3.26), EN (48.59% ± 1.01),   
RSEAL (48.59% ± 3.73) characterized by moderate cytotoxicity. Statistical analysis showed significant differences in cell viability between the materials categorized as moderate and other sealers (p<0.05). Mild cytotoxicity was noticed for   
AH (71.24% ± 7.45), SP (71.39% ± 4.63) and TS (71.39% ± 7.07), what was statistically significant among other sealers from group I (p<0.05). Silicone-based sealers shown no cytotoxicity: GF (143.44% ± 12.84), RSA (127.06% ± 16.57) and AP (95.68% ± 8.62), FL (92.11% ± 12.44) Statistically significant differences, in the human periodontal ligament fibroblast survival, were found between all materials characterized as non-cytotoxic and other sealers (p<0.05).

Comparing „fresh” and „set” sealers, a significantly lower percentage of viable cells in culture was found after set for SP, AH, TS, FL, GF (p<0.05) Opposing and statistically significant differences in cell survival was observed for RSEAL (p<0.001), which turned out to be more toxic immediately after mixing, than after setting. None of the group II materials demonstrated severe cytotoxicity. META (35.71 ± 1.98), SP (45.24% ± 2.71), EN (50.61% ± 4.41), AH (51.33% ± 8.54) ,TS (58.04% ± 7.77) characterized by moderate cytotoxicity. Statistically significant differences were noted between almost all materials with moderate cytotoxicity (except for TS) and other sealers (p<0.05). Mild cytotoxicity was noticed for the RSEAL (67.52% ± 3.57) and FL (70,50% ± 2,45), which turned out to be statistically significant for the other materials (p<0.05), with the exception of RSEAL and TS. No cytotoxicity was shown by: GF (110.49% ± 6.02), RSA (103.08% ± 6.17) and AP (102.41% ± 2.01), which was statistically significant compared to other sealers (p<0.05). GP (111.58% ± 5.06) was considered by statistically significantly lower cytotoxicity compared to RLN (53.14% ± 2.9) (p<0.001). Among cells exposed to the tested materials, only a “fresh” form of sealers (group I) induced a significantly higher level of ROS, in comparison with “set” materials (group II) (p<0.001). The highest level of ROS was observed after cell exposure on “fresh” form of META and EN. No significant differences in the level of oxidative stress was noticed, between each set sealer, as well as between GP and RLN (p> 0.05). The cytotoxicity of sealers based on salicylates (FL, SP), eugenol (EN, TS) and META was mainly associated with necrosis, while in the case of materials based on resins (AH, RSEAL) and AP with apoptosis. No significant differences was found, in induction of apoptosis and necrosis, between GP and RL (p> 0.05).

Conclusions:

1. The cytotoxic effect of root canal filling materials ("fresh" and “set”) on human periodontal ligament fibroblast varied.
2. The highest cytotoxicity, assessed by the MTT test, have metacrylic sealers, zinc oxide-eugenol sealers and resilon. The lack of cytotoxic potential indicated siloxane sealers and gutta-percha.
3. Both forms of sealers, guta-percha and resilon was able to cause cytotoxicity, inducing apoptosis and/or necrosis in human periodontal ligament fibroblast.
4. The toxic potential of Endomethasone N, RealSeal, Sealapex, MTA Fillapex, tested after mixing could be caused by oxidative stress induction in human periodontal ligament fibroblast. The cytotoxicity of other materials seems to be related with other mechanisms.
5. Due to the risk of persistent root canal sealer cytotoxicity, endodontic treatment should be performed in accordance with the principles, that enable to avoid contact between the material and periapical tissues.