Toxicity of Five Root Canal Sealers IN VITRO

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Abstract: Aim of the study was to evaluate cytotoxicity of five root canal sealers at different intervals of time on HEK-293 (human embryonic kidney epithelial cells) and Vero cells (African green monkey kidney epithelial cells). Endomethasone N, EndoREZ, Acroseal, RoekoSeal and AH Plus were mixed according to manufacturer’s instructions. MTT assay was used to evaluate cytotoxicity of freshly mixed and set sealers 0 h, 24 h, 48 h, 72 h and 1 week on target cells differently. Statistical analysis was done using SPSS Version 15.0 (SPSS, Inc., Chicago, IL, USA) statistical software package (p < 0.05). The study concluded that cytotoxicity of freshly mixed sealers start increasing and reaches to maximum at 24-48hr, then starts decreasing and reaches to a stable level at a week interval. Endomethasone N exhibit highest toxicity while EndoREZ was least toxic towards cultured cells. Acroseal, RoekoSEAL and AH-Plus showed intermediate cytotoxicity.

Keywords: MTT assay, root canal sealers, cytotoxicity.

INTRODUCTION

Root canal treatment aims to eliminate infection of the root canal and to completely fill the root canal space in order to prevent apical and coronal penetration of liquids and microorganisms. Currently, most root canals are filled with gutta-percha points in combination with an endodontic sealer. The main function of the sealer is to fill the gaps between the gutta-percha points and the walls of the root canal. The sealer also fills the voids between individual gutta-percha points applied during condensation. It is widely recognized that sealers may come in direct contact with the soft and hard tissues apically for a prolonged period of time and might affect the periapical tissue, if extruded. In such a condition, they could cause not only degeneration of the tissue lying underneath the endodontic sealer but could also delay wound healing.

Therefore, the biocompatibility of the sealers is of primary importance (1). The biocompatibility of different root canal sealers varies considerably (2). Most products exert some toxic effect, when they are fresh and the effect is reduced over time as the concentration of leachable components decreases (3,4). Root canal filling materials have been formulated in an attempt to obtain better physical and biological properties (5).

Endomethasone N (Septodont, St. Maurdes-Fosses Cedex, France) has been introduced as successor of formaldehyde containing sealer to reduce the toxic effects of paraformaldehyde. The cytotoxicity of Endomethasone N is approximately 30-folds lower than a formaldehyde-containing sealer (6).
A sealer containing a single methacrylate (EndoREZ; Ultradent Products, Inc., South Jordan, UT, USA) has been described as well tolerated by connective tissue. The sealer has shown biocompatibility to periapical tissues in subhuman primates (7) and to rat cells and bone tissue (8).

A new calcium hydroxide-based sealer, Acroseal (Septodont, St. Maurdes-Fosses Cedex, France), appears to have lower solubility than other calcium hydroxide sealers, probably because of its epoxy resin component (9). The silicone-based endodontic sealer, RoekoSeal (Colte’ne Whaledent, Langenau, Germany), has been reported to be noncytotoxic (10). The cytotoxicity of fresh epoxy resin-based sealers (eg., AH-Plus; Dentsply DeTrey GmbH, Konstanz, Germany) is well documented (10-13). Such sealers have shown pronounced cytotoxic effects in direct contact test with cultured cells (14). Epoxy resins exhibit severe cytotoxicity immediately after mixing and after time periods up to several hours after mixing. The cytotoxic effects are reduced to control levels after many days to several months (10). However, only minor toxicity has been observed in implantation studies. The dimethylthiazol diphenyltetrazolium bromide (MTT) assay measures cellular metabolic function and is widely used for ex vivo biocompatibility evaluation (15-17).

The aim of the current study was to investigate the cytotoxic effects of eluates of five root canal sealers at different intervals of time on human embryonic kidney cells (HEK-293) and Vero cells (African green monkey epithelial cells).

MATERIAL AND METHOD:

Root canal sealer use were Endomethsone N, EndoREZ, Acroseal, RoekoSeal and AH Plus. Two cell lines HEK-293 (derived from human embryonic kidney cell) and Vero (derived from kidney epithelial cells of an African green monkey, Cercopithecus aethiops) were propagated in minimum essential medium, supplemented with 5% foetal bovine serum (Invitrogen, USA), 2 mmol /L L-glutamine, 100 U/ mL penicillin (Gibco-BRL, USA) and 100 μg/ mL streptomycin at 37 °C in air atmosphere containing 5% CO2 and 95% relative humidity. Cells were passaged by treatment with trypsin-EDTA in phosphate-buffered saline solution. For preparation of sealer extract sealers were mixed according to manufacturer’s instruction and placed in a nonreactive plastic rings (5 mm diameter) and. The groups of samples were placed in contact with cell monolayers immediately after mixing (fresh condition) and after setting (0 h). Specimens of EndoREZ were prepared with light-induced polymerization for 40 s. Another set of test samples was allowed to set for 24 h at 37 °C and 100% humidity (set condition), similarly 48h , 72h and 1 week samples were prepared. Extract of each material was filtered using Millex-GS sterile filter (Millipore, USA) and used as the experimental material. The MTT assay was carried out according to Mossman T (1983) 19. A volume of 20μL of filtered extracts in 200μL medium (control) was added to each well, six replicates for each sample. DMEM (Dulbecco’s Modified Eagle’s Medium) media alone was taken as control. Cells were incubated for 2 h at 37 °C and 5% CO2. The pH of the extracts was checked using pH meter (Decibel instrument, India). Then 20 μl MTT (Sigma Aldrich, USA) was added to each well, followed by 4 h of incubation at 37°C. Medium and MTT were removed. The formazan product was then solubilized by adding 200μl Dimethyl Sulfoxide (Ranbaxy, India) per well, including controls. The plate with cover was left for 15-20 minutes in dark at room temperature. After that plate cover was removed and absorbance in each well is measured including blanks at 540nm in a micro titer plate reader (VERSA max, Germany). The statistical analysis was done using SPSS Version 15.0 (SPSS, Inc., Chicago, IL, USA) statistical software package. p-values <0.05 were considered statistically significant. The values were represented in Number (%) and Mean ± SD. Statistical values for each group of data were subsequently calculated with analysis of variance.
RESULTS:
As depicted in Table 1 and Table 2, Endomethasone N showed highest mean value at 24hr on both cell lines. EndoREZ and RoekoSEAL had highest mean value at 48hr, AcroSEAL at 72 hr, while AH-Plus was most cytotoxic at 24hr on HEK cells and 48hr on vero cells (p<0.05). Data also showed that in all the type (HEK-293 and VERO cells) and point of time (Fresh, 0 hr, 24 hr, 48 hr, 72 hr and 1wk), EndoREZ had the minimum cell inhibition and Endomethasone N had maximum (p<0.001).

Table 1 showing % inhibition/cell cytotoxicity of sealers on HEK cells, where, EN=Endomethasone N, ER=EndoREZ, AC=AcroSEAL and RS=RoekoSEAL

<table>
<thead>
<tr>
<th>Sealer</th>
<th>% Cell Cytotoxicity*</th>
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<tbody>
<tr>
<td></td>
<td>Fresh</td>
</tr>
<tr>
<td>EN</td>
<td>5.46±1.86</td>
</tr>
<tr>
<td>ER</td>
<td>2.63±1.43</td>
</tr>
<tr>
<td>AC</td>
<td>3.40±1.63</td>
</tr>
<tr>
<td>RS</td>
<td>4.43±0.61</td>
</tr>
<tr>
<td>AH-Plus</td>
<td>5.91±1.02</td>
</tr>
</tbody>
</table>

In overall assessment, in HEK-293, Acroseal and RoekoSeal had the minimum intergroup difference while the maximum difference was seen between Endomethasone N and EndoREZ, where as in Vero cells, the minimum intergroup difference was seen between AH Plus and Acroseal while the maximum difference was seen between Endomethasone N and EndoREZ.

DISCUSSION:
The MTT assays are colorimetric methods for quantifying viable cell numbers. The methyltetrazolium ring is cleared by mitochondrial dehydrogenouses in viable cells to formazan, which has a blue colour and can be measured with a spectrophotometer (18). The amount of formazan produced is directly proportional to the total viable cell number over a wide range of cell numbers. The advantages of this method are its simplicity, rapidity, and precision, in addition, it does not require radioisotopes.

Table 2 showing % inhibition/cell cytotoxicity of sealers on Vero cells, where EN=Endomethasone N, ER=EndoREZ, AC=AcroSEAL and RS=RoekoSEAL

<table>
<thead>
<tr>
<th>Sealer</th>
<th>% Cell Cytotoxicity*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh</td>
</tr>
<tr>
<td>EN</td>
<td>2.71±0.93</td>
</tr>
<tr>
<td>ER</td>
<td>2.71±0.79</td>
</tr>
<tr>
<td>AC</td>
<td>2.57±0.79</td>
</tr>
<tr>
<td>RS</td>
<td>2.89±0.28</td>
</tr>
<tr>
<td>AH-Plus</td>
<td>2.48±0.62</td>
</tr>
</tbody>
</table>

In this study, the freshly prepared root canal sealers were placed immediately into medium. Clearly, canal sealer should be tested immediately after mixing and also after a period of time when it is assumed that they have reached their final chemical structure. Root canal sealers are inserted into the mouth in a freshly mixed, incompletely polymerized stage, and thus it is probable that, during a relatively short period after clinical application of the material, local responses are provoked by unreacted or only partially reacted components. After setting, it is possible that potentially toxic constituents may be released from the materials. The difference in toxicity patterns at the various elution times may be related to the degree of setting. This would be
reflected in the rate of component leaching. Thus, the different time extracts might be important to determine long-term cytotoxicity of root canal sealers. The cytotoxicity of extracts of five root canal sealers was evaluated using MTT assay on HEK-239 and Vero cells. Our results agree with previous reports (6,8,10,19-20,21-22) that all materials tested were cytotoxic. In the case of Endomethasone N toxicity decreases with time. This result could be due to release of eugenol which was much slower during initial hours, it peaked after about a day and then declined slowly over several weeks (23). Various researches have been done previously to test the toxicity of Endomethasone but there are limited knowledge regarding the toxicity of Endomethasone N. The cytotoxicity of Endomethasone N is approximately 30-folds lower than a formaldehyde-containing sealer. This sealer is also less cytotoxic than AH26, Tubli-Seal and CRCS (6). Although in present study Endomethasone N is most toxic sealer amongst the sealers used in this study on both cell lines. In this study EndoREZ exhibited limited cytotoxicity which decreases after 48hr. The reason for cytotoxicity may be its Urethane Dimethacrylate (UDMA) content (24-25). Our result agree with Lodiene et al., but not with Bouillaguet et al.

Silicone-based materials have been developed as root canal sealers and laboratory and clinical data are promising (26-27). RoekoSeal is a new silicone-based material. The silicone-based endodontic sealer, RoekoSeal (Colte`ne Whaledent, Langenau, Germany), has been reported to be noncytotoxic (10). It appears to be less cytotoxic than sealers based on methacrylate, zinc oxide-eugenol and epoxy resin (20). However, another study rated the silicone-based sealer equal to an epoxy resin-based sealer in terms of cytotoxicity (28).

Calcium hydroxide sealers are generally characterized as having good cytocompatibility (6,29-33). Acroseal exhibited maximum level of toxicity at 24-72 hours followed by a decrease in toxicity values. The source of the toxicity might be because of the presence of amines in the epoxy base of this material. The mechanism that may explain the inflammatory response regarding AH Plus sealer is the releasing of formaldehyde that has been shown to induce non-neoplastic responses, such as epithelial degeneration and a mixed inflammatory cell infiltration, besides allergic reaction and necrosis of the connective tissue. The toxicity subsided after setting (34,35). However, even after the setting period, toxicity may still exist. So, amines, present in AH Plus composition, which accelerate polymerization, could be also the reason for strong initial toxicity. This is the reason for Minimum value of inhibition in AH Plus group in fresh specimen, followed by a regular rise till 48 hours thereafter a fall till 1 week.

CONCLUSION:

The study concluded that cytotoxicity of freshly mixed sealers start increasing and reaches to maximum at 24-48hr, then starts decreasing and reaches to a stable level at a week interval. Endomethasone N exhibit highest toxicity while EndoREZ was least toxic towards HEK-239 and Vero cells. Acroseal, RoekoSEAL and AH-Plus exhibited intermediate cytotoxicity.

REFERENCES:


