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## RESEARCH ARTICLE

# ASSESSING THE EFFICACY AND CYTOTOXICITY OF FOUR DIFFERENT ROOT CANAL SEALERS

***,[1]Dr. Vanita Gautam and [2]Dr. Hemant Kumar Halwai**

[1]Department of Conservative Dentistry and Endodontics, UCMS College of Dental Surgery, Bhairahawa, Nepal
[2]Department of Orthodontics and Dentofacial Orthopedics, UCMS College of Dental Surgery, Bhairahawa, Nepal

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**ABSTRACT**

**Aim:** to compare the efficacy and cytotoxicity of four different types of sealers including AH plus, Sankin, Tubliseal EWT and Apexit as well as their effect on cytokine release of L929 fibroblasts.
**Materials and Methods:** Here cells were cultured in Complete Medium Culture (CMC) and then divided into two test groups. In group 1, sealers were added to cell culture wells immediately after mixing. In group 2, sealers were added to cell cultures 3 hours after mixing. Cell viability was evaluated by MTT assay after 4, 24 and 168 hours. The amount of Interleukin-6 (IL-6) released in response to the sealers was also evaluated by ELISA technique on fibroblasts after 24 hour period.
**Results:** Significant differences were seen in cytotoxicity in both groups ($P<0.001$). The least cytotoxic sealers were AH Plus and Sankin respectively, whereas Tubliseal EWT showed the greatest cytotoxicity. The highest IL-6 level was observed in Tubliseal EWT and Sankin groups; which was statistically significant ($P<0.001$).
**Conclusion:** AH plus has less cytotoxicity and induces less IL-6 release. Tubliseal EWT has greater cytotoxicity and induces more IL-6 release than other sealers.



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## INTRODUCTION

Various root canal filling materials are being used, and root canal sealers are one of them. The biocompatibility of these materials is important as root canal sealers frequently come in contact with periapical tissues, if extruded during root canal treatment. Ideally a sealer should not hinder tissue repair, but aid or stimulate the reorganization of injured structures. The final outcome of root canal treatment is influenced by these materials1. Different classes of these materials and their products produced during setting reaction have shown variable amount of cytotoxicity (Huang *et al*., 2002; Zmener and Pameijer, 2004). The aim of this study was to compare cytotoxicity of four different types of sealers including AH plus, Sankin, Tubliseal EWT and Apexit as well as their effect on cytokine release of L929 fibroblasts.

## MATERIALS AND METHODS

L929 rat fibroblasts were obtained from microbiology department of the Institute. Cells were grown in complete medium culture (CMC) supplemented with 10% fetal bovine serum (FBS) (Gibco, US) and 100 µg/mL penicillin and 100 µg/mL Streptomycin (Sigma Co., US) under standard cell culture condition (37˚C, 100% humidity, 95% air, 5% $CO_2$). Cells were used in this study after the fourth passage.

**Experimental groups were as follow:**

- Group 1 cells were treated with sealers immediately after the mixing
- Group 2 cells were treated with sealers 3 hours after the mixing

Each group had 5 subgroups including

1. Subgroup1: AH plus (Dentsply, DeTrey, Germany)
2. Subgroup 2: Sankin (Sankin, Kogyo, K.K., Japan)
3. Subgroup 3: Apexit (VivadentSchann Vaduz, Liechtenstein)
4. Subgroup 4: Tubliseal EWT (Kerr Co., Romulus, MI, USA)
5. Subgroup 5: no treatment (control).

For material preparation, tubes with 0.7 internal diameters were cut into lengths of 5 mm each, submerged in distilled water for 24 hours and sterilized at 121˚C for 20 minutes. Materials were

***Corresponding author: Dr. Vanita Gautam,***
Department of Conservative Dentistry and Endodontics, UCMS College of Dental Surgery, Bhairahawa, Nepal.

mixed according to manufacturer's instruction in sterilized conditions under a laminar air flow hood. The tubes were filled with sealers and all procedures were done on vibrator (Delta, Germany). In the first group, the samples were added to cell wells immediately after mixing and in the second group, the samples were added 3 hours after mixing. During these three hours, they were kept under UV-light hood. After 1 h, 24 h, and 7 days of incubation, cell viability was evaluated by 3-(4,5 dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay according to the manufacturer's instruction (Merck Co, Darmstadt, Germany). For inflammatory evaluation of sealers IL-6 level was measured in group two. The cultured suspension was collected and preserved in micro tubes. Micro tubes were cryopreserved at -20˚C during the experiment. For determination of fibroblast cytokine level, IL-6 kit (Bender Med System, Vienna, Austria) was used. Each well of ELISA plate was filled with anti IL-6-monoclonal antibody, and then samples of IL-6 were conjugated with biotin and were added to wells and kept for 2 hours at room temperature. The samples were rinsed with distilled water in order to eliminate unbounded compounds. Streptavidin HRP was added in order to be bonded with conjugated biotin-interleukin. Rinsing was carried out again after 1 hour at room temperature and samples were assessed at 450 nm. The quantity of staining was directly related to the concentration of IL-6.

## RESULTS

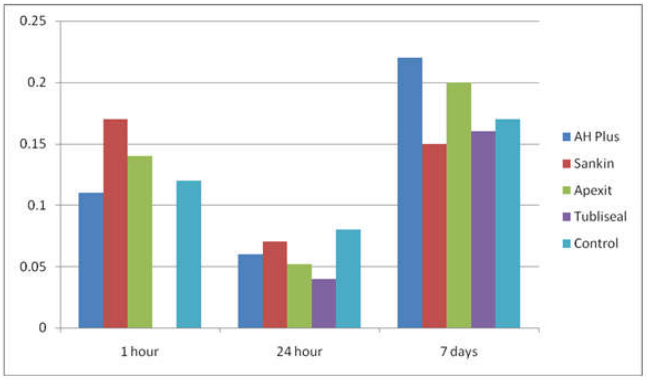
Significant differences were observed in cytotoxicity of different sealers in both groups (P<0.001). In group 1 (fresh specimens), the lowest cytotoxicity belonged to Sankin with a minor and insignificant increase. In group 2 (set specimens) the lowest cytotoxicity belonged to AH Plus which increased after 24 h, decreased after 7 days and finally reached the control group level. Regarding the production of IL-6, significant differences were seen among the sealers (P<0.001). The highest level was observed in Sankin followed by Tubliseal EWT, and the lowest level was achieved in AH Plus group

**Table 1. Comparison of fibroblast IL-6 production after exposure to 4 different sealers in group2 (set specimen)**

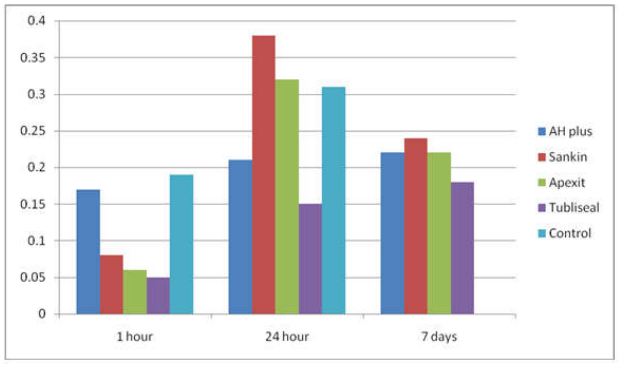
| Sealers | Mean ± SD |
|---|---|
| AH plus | 0.081±0.041 |
| Sankin | 0.060±0.002 |
| Apexit | 0.052±0.001 |
| Tubliseal | 0.065±0.004 |
| Control | 0.092±0.053 |

## DISCUSSION

Fibroblasts are the major cells of connective tissue and have the ability to produce and protect connective matrix. During the inflammatory phase, the fibroblasts stimulated by inflammatory cytotoxin and bacterial products take part in connective tissue lysis (Seltzer and Bender, 2002). The rat L929 fibroblasts were selected for this study as this cell line is easy to prepare and culture without the individual difference of primary cells (Azar *et al*., 2000; Koulaouzidou *et al*., 1998; Oztan *et al*., 2003). In this study we found that the cytotoxicity of Tubliseal remained high after 1 week and increased with time, which is in accordance with the study conducted by Ersev and colleagues (1999). Another study conducted by Leonardo *et al.* (2000) evaluated the cytotoxic effects of Apexit, CRCS, and Sealapex on rat's peritoneum macrophage morphology and concluded that Apexit has the highest cytotoxicity; interestingly our analysis showed the same results. Camps *et al.* (2003) in an investigation of cytotoxicity of AH Plus, Cortisonol and Sealapex on L929 rat's fibroblasts concluded that AH plus had the lowest cytotoxicity from the first day up to the third day; our study on the other hand demonstrated the lowest cytotoxicity on the 7$^{th}$ day. Haung *et al.* (2002) assessed the cytotoxicity of set sealers (AH26, AH plus, N2 canals, Endomethasone, Sealapex) on human PDL and V19-Hamster cells and concluded that AH plus causes the inhibition of PDL cells' growth from the first day up to the third day. The cytotoxicity chiefly decreased from the second day. Their results are in agreement with our observations. For IL measurement, only set samples were chosen as release of sealer contents into culture medium could increase the turbidity of the samples and interfere in ELISA reading. A similar study was conducted by Menden *et al.* (2003) wherein they tried to evaluate inflammatory and cytotoxic effects of freshly mixed and set sealers (Endofill, Sealer EWT, Kerr pulp canal) on macrophage cells. They demonstrated that both groups had the same inhibiting activity on cells growth and that the inflammatory effects (release of IL-2) were not affected by sealers.




**Graph 1. Comparison of optical density (OD) of viable cells after exposure to 4 different sealers in first group (fresh specimen)**




**Graph 2. Comparison of optical density of viable cells after exposure to 4 different sealers in second group (set specimen)**

### Conclusion

The distinct cytotoxicity of some specimens observed in this study may appear alarming. Our current finding mostly

corroborate with already reported toxicity profile of the sealers investigated in the study. Whilst biocompatibility is a desirable quality, extrapolations to the clinical situation must be made with caution, as the results of such in vitro toxicity tests may not correlate with the in vivo response.

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