

## BIOLOGICAL EFFECTS OF ENDODONTIC MEDICATION ON HUMAN FIBROBLAST CELL CULTURE

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The aim of this in vitro study was to determine the cytotoxic effect of endodontic medication in the treatment of apical periodontitis. *Materials and Methods:* The toxicity of four antibacterial products (Cresophene, Septodont, Ledermix, Riemser, R4, Septodont, Calxyl, OCO Präparate.) commonly used as endodontic dressings, has been tested on a stabilized human diploid cell line of fibroblasts ICP-23, similar to human diploid cell lines belonging to ATCC (American Tissue Culture Collection). The outcome of the study, that run 96 hours, was expressed as a mortality test. *Results:* Statistic analysis of the mortality of a fibroblasts cell line (ICP-23) induced by different endodontic dressings highlights the lowest cytotoxicity for Ledermix and highest levels for Calxyl and Cresophene. In case of Ledermix, the cell mortality raised after 96 hours only with 50%, as compared to Cresophene and Calxyl, approximately twice. R4 had an intermediate value. *Conclusions:* Some proprietary antimicrobial products, commonly used as endodontic dressings, could elicit in vitro lethal cytotoxic responses in fibroblasts of the cell line ICP-23, owing to their composition, as antiseptics, like chlorphenol and tymol (Cresophene) or because of their high alkalinity - up to pH 11-12 (Calxyl).

*Key words:* endodontic medication, cytotoxicity, cellular mortality.

### INTRODUCTION

Chronic apical periodontitis is an infectious disease, caused by bacteria colonizing the necrotic endodontic system. In order to achieve an optimal outcome of the endodontic treatment, the antibacterial medication is still mandatory in case of apical periodontitis, although being considered as having a subordinate role, facing the root canal cleansing and shaping<sup>1</sup>. It's targets are maintaining of the disinfection conditions between the treatment appointments, as well as triggering the re-mineralization process in the apical territory<sup>2</sup>.

Dental antibacterial products used in endodontics must be biocompatible, to minimize the adverse effects on periodontal tissue and on alveolar bone, induced by direct or indirect contact

with the endodontic medication. In vitro tests have been recommended for evaluating the cytotoxicity of endodontic substances and a number of model systems, using established cell lines, as well as primary cells, have become available for screening purposes<sup>3,4</sup>.

By means of in vitro testing, effect of the endodontic substances and materials on cellular metabolism is determined and analyzed. Cellular morphological alterations and viability, as a direct expression of the toxicity of the investigated products, are also investigated<sup>5,6</sup>.

In this study, the cytotoxicity of four different antimicrobial products, used in endodontic treatment of apical periodontitis was evaluated on a fibroblast cell line (ICP-23). The results are expressed as mortality test.

## MATERIALS AND METHODS

Cytotoxicity of the investigated products was assessed on a cell line of stabilized fibroblasts, called ICP-23. This cellular culture (obtained and stabilized in the Cellular Substrata Laboratory at the Cantacuzino Research Institute, Bucharest, Romania) is a human cellular population of embryonic origin, fibroblast type, normal from the morphophysiological point of view. The ICP-23 fibroblasts line has no contaminants, nor foreign genetic information and it has a limited in vitro life-span, all these characteristics being specific to a normal diploid cellular line.

ICP-23 line is similar to human diploid cell lines, MRC and WI belonging to ATCC

The cell line, maintained by serial passages at the rate of dispersion of 1:2, was prepared in Barski tubes. Cells were exposed for 96 hours to test samples and the cytotoxicity was evaluated by the measurement of cell mortality parameter at every 24 hour interval.

Of all tested substances, Ledermix and Calxyl are presented as paste, which created difficulties as far as dissolving them in the growing medium. The products under the form of a paste were dissolved in sterilised phosphate-buffered saline.

The commercial products under the form of a liquid (Cresophene and R4) were tested undiluted.

The fibroblasts were incubated in 5 vials (2 ml testing tubes) at  $7.39 \times 10^6$  cel/cm<sup>2</sup>. After the growing medium was sucked in, the fibroblasts were incubated for 96 hours in a non-seric medium with the tested substances, at 37°C. The quantities of substances used were 0.05 ml for a 2 ml cellular culture vial from the specified line. Sterilizing filtering was employed.

We obtained 5 growing environments lots with 7.20–7.30 pH. A lot represented the sample lot or control group.

Assays for death cells were performed, using the cellular suspension prepared by detaching the cellular layer with trypsin, resuming in a known environment volume, coloring with trypan blue and counting in the haemocytometer. Cytotoxicity was determined from the number of dead cells in the presence of endodontic agents, relative to the initial number of cells in the investigated area.

A number of four antibacterial products commonly used as endodontic dressings have been tested :

1. Cresophene (Septodont)  
Dexamethasone.....0.10 g  
Parachorophenol.....30.00 g  
Thymol.....5.00g  
Excipient.....100.00 g

2. Ledermix (Riemser)  
Demeclocycline calcium ..30.21g  
Triamcinolone acetonide.....10 g  
Excipient qs.....100.00 g

3. R4 (Septodont)  
Chlorhexidine digluconate( 20%)...0.2 g  
Denaturated ethyl alcohol.....40.00 g  
Excipient q.s.....100.00 g

4. Calxyl (OCO Preparate)  
Calcium hydroxide.....23.00 g  
Barium sulphate.....27.00 g

The cytotoxic effect of the tested substances was also investigated, by means of the microscopic examination of the cellular layer.

## RESULTS

All materials were cytotoxic in ICP-23 cell line; the results indicated that the mortality rate in the presence of investigated substances was significantly higher compared with that of control group ( $p < 0.05$ ). However, statistic analysis (Fischer and *t* – Student tests) of the mortality of a fibroblasts cell line (ICP-23) induced by different endodontic dressings highlights the lowest cytotoxicity for Ledermix and the highest for Calxyl and Cresophene. In case of Ledermix the cell mortality raised after 96 hours only with 50% as compared to calcium hydroxide(Calxyl) and Cresophene, approximately twice. R4 had an intermediate value. Cell death rates are shown in Table 1 and Figure 1.

Table 1

Cell mortality (%) induced by endodontic dressings for cell line ICP-23.

Endodontic antibacterial Products	24 hours	48 hours	72 hours	96 hours
1. Cresophene	6.77	7.24	9.18	10.43
2. Ledermix	2.42	3.12	4.60	5.13
3. R 4	4.48	5.26	6.81	9.62
4. Calxyl	8.64	9.09	10.2	11.95
5. Control	1.30	2.72	3.90	4.87

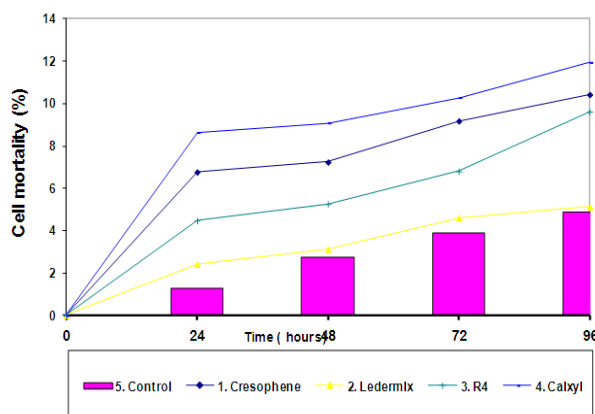


Figure 1. Cell mortality evolution. The ICP-23 line in the presence of all tested substances.

Contiguous and in connection with the mortality rates, the microscopic examination points out the following aspects: rounded cells, detached from the cellular monolayer and being in suspension were observed (Figure 2). This represents another way to assess the cytotoxic effect of the tested endodontic products. Microscopic images also revealed cellular morphological alterations which are degenerative: cellular membrane disintegration (Figure 3), the presence of the pathological pseudopodium, the nuclei and nucleolic changes (Figure 4).

Also, a significant number of dead cells, associated with a clear-cut delimitation between the affected and the intact cells (Figure 5).

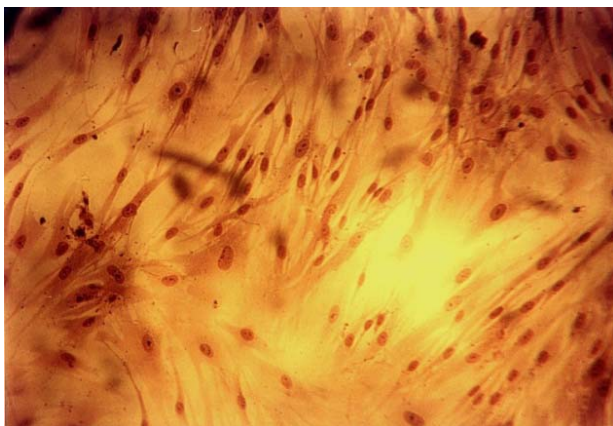


Figure 2. Fibroblasts (ICP-23 line) in contact with Ledermix, at 24 hour mark (MGGX40 stain). Round cells in suspension, detached from the cellular monolayer.

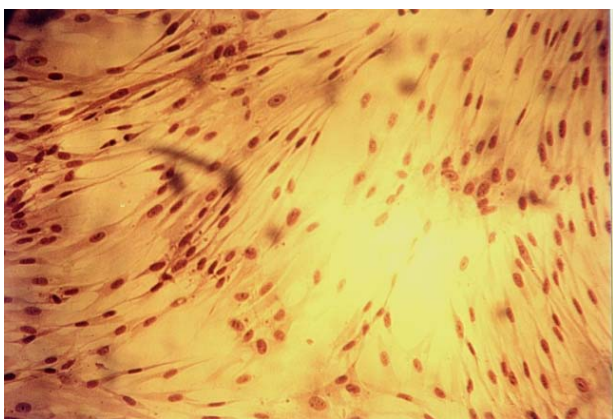


Figure 3. Fibroblasts (ICP-23 line) in contact with Ledermix, at 96 hour mark (MGGX40 stain). Degenerative cellular alterations in association with an increased number of detached cells.

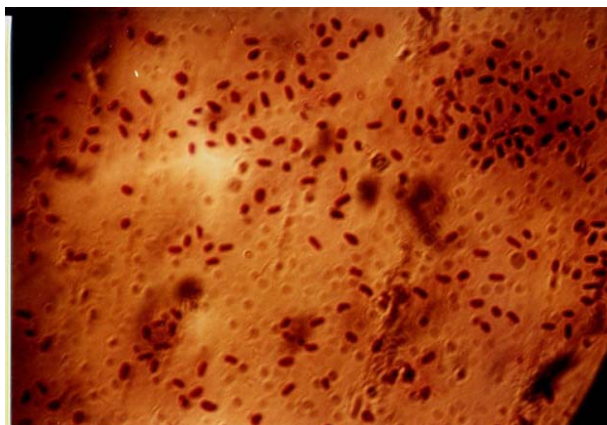


Figure 4. Fibroblasts (ICP-23 line) in contact with Calxyl at 24 hour mark (MGGX40 stain). Degenerative cytoplasmic and nucleolar changes with cell membrane disintegration.

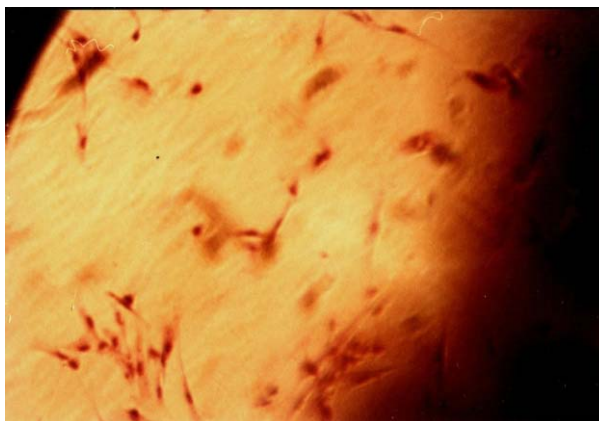


Figure 5. Cellular alterations in fibroblasts (ICP-23 line) after contact with Calxyl at 96 hour mark (MGGX40 stain): large percentage of dead cells as a result of highly cytotoxic effect.

## DISCUSSIONS

*In vitro* biocompatibility tests have been developed to simulate and predict biological reactions to antimicrobial medications, when they are placed into the endodontic system, as root canal medication. These methods offer a relatively inexpensive way to evaluate the cytotoxic potential of dental materials<sup>7</sup>.

It has been well established, that an inflammatory apical reaction may be elicited by an endodontic dressing<sup>8,9</sup>. The aim of this *in vitro* study was, to find out to what degree a usual antibacterial dressing can induce a harmful response in a human cell line.

In the present study, the human fibroblasts ICP-23 cell line was used to study the cytotoxicity of endodontic dressings, because of their well-define

culturing characteristics in experimental condition and their relevance as an *in vitro* system, for screening tissue and cellular biocompatibility. In addition, the selection of a permanent cell line was desirable as they are easily maintained in culture.

The quantitative measurement of death cells is a test that can easily quantify the cellular sensibility level for a certain substance. The test is considered significant when the mortality rate in monolayer is at least 10% of the cells in the investigated area.

This detectable effect is always anticipated by the signs of cellular distress, which are more difficult to quantify.

The results of the cytotoxicity tests for the ICP-23 fibroblasts line, to established a toxicity scale are: Calxyl has the highest cytotoxic effect, expressed by the highest rate of mortality, in cell culture, at every measurement, during the 96 hours test. This paste owes its cytotoxic effect to high alkalinity (pH 11–12), with a necrosis effect, when it touches the vital tissue<sup>10, 11</sup>. This is in apparent contradiction with the fact, that calcium hydroxide is well tolerated clinically, stimulating the defensive mechanisms at apical level. Currently, calcium hydroxide is recognised as one of the most effective antimicrobial dressings available for the purposes of endodontic treatment<sup>12</sup>.

During our investigation, Calxyl was dissolved in the culture growing medium, and even so, the product was the most cytotoxic material. In case of Calxyl the cell mortality was significant after 96 hours.

The cellular degenerative changes were present in the first 24 hours after the experiment had begun and they were continuously aggravated during the entire experiment.

The results of the present study show a relatively high toxic effect of Cresophene on tested fibroblasts. This antiseptic exerts his cytotoxicity to his components: thymol and parachlorphenol. In decreasing order of cytotoxic effect followed R4(chlorhexidine digluconate). Chlorhexidine is effective as an irrigation solution and is also used as an endodontic dressing. This synthetic cationic bisbiguanide is highly efficacious against several gram-positive and gram-negative oral bacterial species, but despite claims of biocompatibility, chlorhexidine is cytotoxic when in direct contact with human cells. Several studies found chlorhexidine to be uniformly toxic to both cultured human cells and microorganisms<sup>13</sup>.

Ledermix, wich presented the weakest cytotoxicity in the investigated lots, is a

chemotherapeutic product, with selective and specific toxicity over pathogenic germs, without injuring the cells of the host organism. This explains it's good behaviour in *in vitro* biocompatibility tests. Ledermix is an effective intracanal medicament for the control of postoperative pain associated with acute apical periodontitis<sup>14</sup>.

Simultaneously with quantitative death cell determinations, the microscopic examination emphasized the degenerative nature of cellular morphologic changes, which increased continuously during the experiment and finally leading to cellular death, forming a clear-cut distinction between the affected and the intact cells. This is especially the case where the cytotoxic effect was higher.

The results of this present study are in agreement with the data in the expert medical literature. It is well known that antiseptics are chemical substances with non-specific and non-selective toxic action, resulting in the direct alterations of the tissue, together with cellular destruction<sup>15-17</sup>. The effect is exerted to both microorganisms's cells and to the cells of the host organism.

Endodontic medications applied to root canals can circulate through the body in the bloodstream, after penetrating the dentin or passing through apical foramen. Therefore, these products should have low cytotoxicity and high safety. From this point of view, cellular culture testing can serve as a „pre-screen” in order to predict the potential toxic and irritative effect *in vivo*. It is difficult to predict accurately which is the *in vivo* behaviour of the fibroblasts in the apical area and further investigations of biocompatibility are suggested.

## CONCLUSIONS

The present study results showed that all endodontic antibacterial products studied have significant biological risks, as the ICP-23 fibroblasts were extremely sensitive to all tested substances. The test results on human fibroblasts (ICP-23 line), regarding the citotoxicity of endodontic dressings are: Calxyl, has the highest cytotoxic effect; followed by, in decreasing order of the mortality rate, Cresophene and R4. The lowest mortality rate was determined by Ledermix, the most biocompatible investigated intracanal medicament.

## REFERENCES

1. Athanassiadis, B., P. V. Abbott and L. J. Walsh. The use of calcium hydroxide, antibiotics and biocides as antimicrobial medicaments in endodontics. *Aust Dent J* 2007, 52 (1 Suppl): S64-82.
2. Ballal, V., M. Kundabala, S. Acharya and M. Ballal. Antimicrobial action of calcium hydroxide, chlorhexidine and their combination on endodontic pathogens. *Aust Dent J* 2007, 52 (2): 118-2.
3. Washington JT, Schneiderman E, Spears R, Fernandez CR, He J, Opperman LA. Biocompatibility and osteogenic potential of new generation endodontic materials established by using primary osteoblasts. *J Endod*. 2011 Aug;37(8):1166-70.
4. Barnhart, B. D., A. Chuang, J. J. Lucca, S. Roberts, F. Liewehr and A. P. Joyce. An in vitro evaluation of the cytotoxicity of various endodontic irrigants on human gingival fibroblasts. *J Endod* 2005, 3 (8): 613-5.
5. Zoufan K, Jiang J, Komabayashi T, Wang YH, Safavi KE, Zhu Q. Cytotoxicity evaluation of Gutta Flow and Endo Sequence BC sealers. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2011 Nov; 112(5):657-61.
6. Hauman, C. H. and R. M. Love. Biocompatibility of dental materials used in contemporary endodontic therapy: a review. Part 1. Intracanal drugs and substances. *Int Endod J* 2003, 36 (2): 75-85.
7. Willershausen I, Callaway A, Briseño B, Willershausen B. In vitro analysis of the cytotoxicity and the antimicrobial effect of four endodontic sealers. *Head Face Med*. 2011 Aug 10; 7:15.
8. Eldeniz AU, Mustafa K, Ørstavik D, Dahl JE. Cytotoxicity of new resin-, calcium hydroxide- and silicone-based root canal sealers on fibroblasts derived from human gingiva and L929 cell lines. *Int Endod J*. 2007 May; 40(5):329-37.
9. N.J.A. Souza, G.Z. Justo, C.R. Oliveira, M.Haun. Cytotoxicity of materials used in perforation repair tested using the V79 fibroblast cell line and the granulocyte-macrophage progenitor cells. *Int Endod J* 2006, (39): 40-47.
10. Mohammadi Z, Dummer PM. Properties and applications of calcium hydroxide in endodontics and dental traumatology. *Int Endod J*. 2011 Aug; 44(8):697-730.
11. Silva EJ, Herrera DR, Almeida JF, Ferraz CC, Gomes BP, Zaia AA. Evaluation of cytotoxicity and up-regulation of gelatinases in fibroblast cells by three root repair materials. *Int Endod J*. 2012 Sep; 45(9):815-20.
12. Sirén EK, Haapasalo MP, Waltimo TM, Ørstavik D. In vitro antibacterial effect of calcium hydroxide combined with chlorhexidine or iodine potassium iodide on *Enterococcus faecalis*. *Eur J Oral Sci*. 2004 Aug; 112(4): 326-31.
13. Mohammadi, Z. and P. V. Abbott. The properties and applications of chlorhexidine in endodontics. *Int Endod J* 2009, 42, (4): 288-302.
14. Willershausen B, Willershausen I, Ehlers V, Azaripour A, Briseño B. A prospective clinical trial on the influence of a triamcinolone/demeclocycline and a calcium hydroxide based temporary cement on pain perception. *Head Face Med*. 2012 Mar 13;8:9.
15. G. Susini, I. About, L. Tran-Hung. Cytotoxicity of Epiphany and Resilon with a root model. *Int Endod J* 2006, (39): 940-944.
16. Y. Ho, F.M. Huang. Mechanisms of cytotoxicity of eugenol in human osteoblastic cells in vitro. *Int Endod J* 2006, (39): 389-393.
17. Silva EJ, Accorsi-Mendonça T, Almeida JF, Ferraz CC, Gomes BP, Zaia AA. Evaluation of cytotoxicity and up-regulation of gelatinases in human fibroblast cells by four root canal sealers. *Int Endod J*. 2012 Jan; 45(1):49-56.