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ORIGINAL PAPER

Evaluation of Biocompatibility of Root Canal Sealers on L929 Fibroblasts with Multiscan EX Spectrophotometer

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ABSTRACT

Introduction: The purpose of the current study was to estimate the biocompatibility of endodontic sealers with different bases on L929 mouse fibroblasts permanent cell line using Multiscan EX Spectrophotometer. **Materials and Methods:** Endodontics sealers used in this study were GuttaFlow (Roeko) silicone based sealer, AH plus (De Tray-DENTSPLY) epoxy resin based, Apexit (Vivadent) calcium hydroxide based and Endorez (Ultradent) methacrylate based sealer. Sealer were tested trough time, freshly mixed 24 h, 48h and 7 days after setting. Biocompatibility was determinate on permanent cell lines L929 mouse fibroblasts trough cytotoxicity using MTT assay. Level of absorption was measured with multi scan EX spectrophotometer on length 420-600 nm. **Results:** Sealer based on calcium hydroxide Apexit Plus, GuttaFlow silicone based sealer and AH plus epoxy resin based sealer, have shown a low cytotoxicity through the all periods of time on culture of L292 mouse fibroblasts. Methacrylate based sealer, Endorez showed moderate cytotoxicity when freshly mixed and after 7 days. After 24 hours the visibility of the cells was 74,0% and after 48 hours 65,1%. which is slightly cytotoxic. **Conclusions:** According to results of this study there is a statistically significant difference among the groups p<0,05 for all the tested sealers. Apexit Plus, GuttaFlow and AH plus can be considered as biocompatibile. EndoREZ sealer which is based on methacrylate, after 7 days shows 50,1% of visible live cells which is considered as moderate cytotoxicity..

Key words: root canal sealers, biocompatibility, multiscan EX Spectrometer.

1. INTRODUCTION

Obturation of the endodontic space is one of the most important steps in endodontic therapy. Endodontic filling materials stays in very close contact with soft periodontal tissue in apical region for a long period and there interaction is expected. Presently, there are variety of obturation techniques and materials used for root filling, but the most preformed technique is combination of gutta-percha with an endodontic sealer. Endodontic sealer should have good physical properties and biological compatibility (1). One method of testing the biocompatibility of root canal sealers is to use an *in vitro* model to determine the cellular response (1).

2. MATERIAL AND METHODS

2.1. Cell Culture

The mouse fibroblasts which were used in the experiment are manufactured frozen mouse fibroblasts L929 (Cat. No. 85011425 LOT09B006 European Collection of cell Culture). The cells were dissolved in the water bath on the temperature of 37^0 C and then washed up by a heated minimal essential medium, supplemented with 10% fetal calf serum and 1% penicillin, streptomycin and neomycin in order to completely remove the cryoprotective DMSO-dimethyl sulfoxide (Sigma, Prod. No. D2650). Cells were placed in to flasks with the cell medium (MEM+ 10% foetal calf serum + 1% penicillin, streptomycin, and neomycin) and left in the incubator on 37^0 C and 5% CO2. The cells were microscopically monitored every 24 hours changing the medium. When the cells in the flasks multiplied and conflated and when the absence of any bacteria or fungus was determined the splitting was initiated. The medium was taken out and the cells were washed with PBS which does not contain Ca2+/Mg2+(Prod. No. D8537). Then trypsin EDTA (Prod. No. T4049) was added 1 ml per 25 cm2. The flasks were slightly shaken and put into the incubator for 10 minutes. After that the cells were microscopically watched, in order to make sure that they had split from the base and that they were floating. Then the cells were suspended with a small quantity of cell medium in order to activate trypsin, it was taken 100-200 µl and then the counting of the cells started. When a certain number was reached the cells were seeded in plates with 96 wells.

2.2. Preparation of Sealer Specimens

Root canal sealers were prepared according to the manufacturer's recommendation. The sealers were then placed into sterile, cylindrical Teflon moulds which had 4 mm diameter and 2 mm height. Four samples of each sealer from first group were immersed in medium, immediately after setting. Specimens from the second, third and fourth group were stored in humid environment at 37 °C for 24 hours, 48 hours and 7 days and then taken to the cell culture medium for testing.

2.3. Preparation of Extraction Medium

Extraction medium was prepared in cell culture medium as 1.25cm²/ml. It was the proportion of the surfaces of the specimens and the volume of the medium. The petri dishes, in which the extracts were stored, were incubated for 24 hours at 37° C.

The specimens were removed and the extracts were sterile filtered using Millex-GS sterile filter . Undiluted extracts were used for the testing.

2.4. Cytotoxicity Test

The MTT assay was used for determination of a sealer cytotoxicity on the permanent cell lines L929 mouse fibroblasts. MTT test 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide solution is used for measuring metabolic function and it is widely applied in vitro evaluation of cytotoxicity of dental materials (1, 2, 3). The advantages of this method are its simplicity, quickness and reality. It also does not require radioisotopes.

After the L929 mouse fibroblasts were incubated with extraction (test) medium for 24 h, medium was removed and 10 µlt MTT Dimethylthiazol diphenyltetrazolim bromide was added and then incubated for 4 hours on 37°C.

After incubation MTT was aspirated. Formazan products were dissolved in 0,1 ml HCl (0,04 ml L-1) in isopropanol. The fibroblasts were then placed into Multiscan EX spectrophotometer of measuring the level of absorption of the cells on length 420-600 nm.

Multiskan EX photometric 96-well microplate absorbance reader, including an internal software. It is especially adapted for ELISA and for any colorimetric assays that require detection in the visible range, from 400 to 750 nm, for example tetrazolium-based cell viability assays, such as the MTT or the MTS assays.

The original rates of the tested cultures are expressed in percentages which were obtained from the control medium.

Absorbing rates which were obtained by the control, were considered 100% visible (colored live cells). Determination of cytotoxicity was based on relative visibility of the colored live cells as follows: >90% visibility - not cytotoxic 60-90% visibility -slightly cytotoxic, 30-59% visibility–moderately cytotoxic and <30 % visibility extremely cytotoxic.

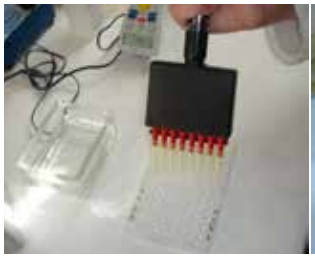


Figure 1. *MTT adding*

Figure 2. *Multiscan EX spectrophotometer*

3. RESULTS

Tables 1 and 2 show descriptive statistics of total L929 mouse fibroblasts. Graph 1 and 2 presents values of surviving fibroblasts cells through time.

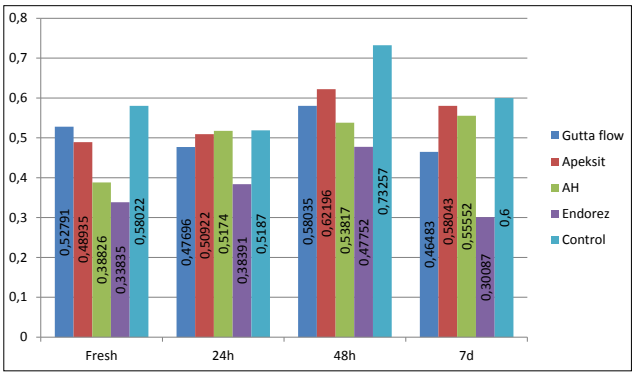
L929 fibroblasts					
	N	Mean	Std. Deviation	Minimum	Maximum
Apeksit	92	.550239	.1761527	.2520	1.0020
AH	92	.500924	.1752526	.1610	.7930
Gutta flow	92	.512511	.1721500	.1880	.9710
Endorez	92	.375163	.1597099	.1370	.8800
Kontrola	92	.607870	.2191576	.2840	1.2090
Period	92	2.50	1.124	1	4

Table 1. *Total descriptive statistics for L929 fibroblasts with different sealers*

Test Statisticsa,b					
	Apeksit	AH	Gutta flow	Endorez	Kontrola
Chi-Square	12.499	13.931	6.955	21.612	13.356
df	3	3	3	3	3
Asymp. Sig.	.006	.003	.073	.000	.004

a. Kruskal Wallis Test
b. Grouping Variable: Period

Table 2. *Testing ranks by X2 test*



Graph 1. *Values of surviving fibroblasts cells through time*

Descriptive Statistics					
	N	Mean	Std. Deviation	Minimum	Maximum
Apeksit %	92	94.5678	23.83266	40.45	195.16
AH %	92	86.2846	28.71265	31.93	236.56
Gutta flow %	92	88.3054	25.61952	44.83	181.84
Endorez %	92	65.4635	26.17272	18.94	170.32
Period	92	2.50	1.124	1	4

Table 3. *Descriptive statistics for the total value of L929 fibroblasts expressed in %*

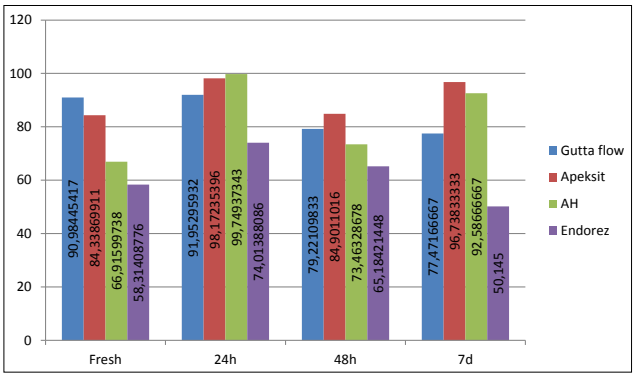
Test Statisticsa,b				
	Apeksit %	AH %	Gutta flow %	Endorez %
Chi-Square	7.705	33.624	9.078	19.431
df	3	3	3	3
Asymp. Sig.	.053	.000	.028	.000

a. Kruskal Wallis Test
b. Grouping Variable: Period

Table 4. *Testing ranks of fibroblasts expressed in percents by X2 test*

4. DISCUSSION

In the last phase of endodontic therapy it is necessary to achieve two goals. First is the complete closing of the root canal, both coronary and apically, and secondly to enable the process of healing of the periodontium. The healing of the periodontium will happen when the biological conditions are enabled. It can be achieved only by a good 'hermetic' closing of the root canal and all the other lateral canals and apical deltas with an appropriate materials, which also have to be



Graph 2. *Values of surviving cells in L929 fibroblasts through time expressed in %*

biocompatible. Biocompatibility is even considered to be the prime condition for a good healing of the periodontium (4).

Sealers which are based on calcium hydroxide have been researched a lot and experimented with in the field of biocompatibility due to the fact that the anti-microbes and regenerating effects of Ca hydroxide has proved to be significantly important (5). Findings of Beltes et all 1995, Vajrabhaya and Sithisarn 1997, Geurtsen et all (2001), Miletic et all (2000), Schwarze et all (2002) (1, 6, 7, 8, 9, 10) shows that sealer based on calcium hydroxide are biocompatible and called " biological sealers".

In this research a sealer based on Ca hydroxide Apexit Plus also has shown a low cytotoxicity through the periods of time with mouse fibroblasts L292 and it can be said that it is biocompatible.

A sealer based on silicon Gutta Flow has also a significant biocompatibility in this research. On the permanent mouse fibroblasts L929 in a fresh condition the visibility of live cells is 90,9%. After 24 hours it is 98,1%, after 48 hours it is 79,2% and after 7 days the visibility of live cells is 77,4%.

Freshly mixed AHA has the lowest number of visible cells 66,9% and it visibility increases as the time passes. After 24 hours it is 99,7 %, after 48 hours it is 73,4 % and after 7 days it is 92,5 %. However the decrease of visible live cells is still within the border lines, but we can say that the results agree with the research by Bouillaguet et all in 2006 who claims that the cytotoxicity of this sealer increases as the time passes (11).

EndoREZ, freshly mixed, has a live cells visibility of 58,3% which classifies it as moderately cytotoxic. After 24 hours the visibility of the cells is 74,0% which is slightly cytotoxic. After 48 hours it is 65,1% and after 7 days the percentage of live cells is 50,1%–moderately cytotoxic. According to Kim et all (2010), this sealer is well tolerated by both connective tissues and bone tissue. Sealer also has minimal cytotoxic effect, both freshly prepared and after setting (12). These findings were not supported by Bouillaguet et all (2006) and Scarparo et all (2009) (11, 13). Cited findings indicate that EndoREZ causes more severe and longer inflammation response in subcutaneous connective tissue in rats. Furthermore, authors proclaim this sealer cytotoxic, with it's toxicity raising over time (11, 13, 14, 15).

5. CONCLUSION

Biocompatibility of the endodontic sealer is one of the basic conditions for a successful endodontic treatment and healing of the periodontium.

With the L 929 permanent cell line mouse fibroblasts the tests has proved that there is a statistically significant difference among the groups of the tested sealers ($p<0,05$). Methacrylate based sealer EndoREZ , shows a 50,1% of visible live cells after 7 days, which is considered as moderate cytotoxicity.

CONFLICT OF INTERESTS: NONE DECLARED.

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