Original Article

Cytotoxicity Assessment for Root Canal Sealers

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Abstract

The purpose of this experimental study was to evaluate the cytotoxicity of four root canals sealer at different times Extract of set root canal sealer (watch's, Endomethazon, refill, and Ditket)were added to cultured vero cells. The cytotoxic effect due to these extracts was evaluated after 24h,48h, and 72h by the Elisa reader which measures the optical density of each well at the end of the experimental time. The result showed that all sealers were cytotoxic. Watch's sealer was significantly less toxic than other sealers on 1st and 2nd experimental days The highest cytotoxic effect appeared with Endomethazon then Dorifill and Diket On the 3rd experimental day no significant cytotoxic effects were found between all saler and statistically all sealer had lost 36-45 percent of there cytotoxicity.

Keywords: Root Canal Sealers, Endodontic Treatment, Biocompatibility of Material.

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INTRODUCTION

The final stage of endodontic treatment is to fill the entire root canal system and all its complex anatomic pathways completely and densely with a nonirritating hermetic sealing material (Berman & Hargreaves, 2019). Incomplete obturation of the root canal system is considered to be the most frequent cause of failure in endodontic treatment (Furtado et al., 2021). Radio-isotope studies, fluorescent assays, dye penetration investigations, and scanning electron microscope studies have shown that filling materials, such as gutta-percha and silver points, do not adhere to the walls of root canals and that the sealing capacity of these filling materials is improved significantly when they are used in conjunction with root canal sealer (Adhikari and Jain, 2018). Since sealers are used extensively in endodontic treatment, and often some of the material is extruded into the periapical tissues, it is important to determine the effect of sealers on vital tissues. The immediate effect of extrusion of any material into vital periapical tissue is inflammation which is sometimes associated with pain. It is reasonable to expect that the more sealer is cytotoxic the more likely it could initiate an inflammatory response (Shashirekha et al., 2018).

LITERATURE REVIEW

The aim of filling the root canal system is to prevent recontamination by the micro-organism, either From those microbes left in the canal after preparation or from new invaders from the coronal access or lateral communication the root, the filling should therefore be able to destroy residual micro-organisms and adapt adequately to root canal walls to prevent their passage or growth. It should also prevent leakage of molecules capable of supporting microbial growth or initiating a peri apical defense response.

The degree of sealer required is dictated by the smallest molecules capable of initiating and sustaining peri apical inflammation (Tan et al., 2015) record a higher proportion of failure in cases where the radiographs reveal poor adaptation and shrinkage of root filling (Kunert and Lukomska-Szymanska,, 2020) found that the most common cause of failure in endodontic treatment was a poor filling canal, in agreements (Tabassum and Khan, 2016) attributed more than 90/ of failure to incomplete sealing. To minimize the incidence of local and /or systemic side effects. the bio-compatibility of all endodontic materials. should be investigated by various in vitro and Vivo tests before clinical application. The biocompatibility testing includes .genotoxicity.mutagenicity, cytotoxicity, and histocompatibility.

Tissue culture cytotoxicity assay

In general cytotoxicity tests measure the effect of material on(1)cell number or growth (2)integrity of cell membrane (3)biosynthesis or enzymes activity (4)the genetic material of the cell. Cytotoxicity test by tissue culture technique has the advantages of1(1)testing for a specific function of cell metabolism in isolation from other events(2)screening a large number of samples quickly and inexpensive (3)quantifying results (4) giving greater sensitivity to toxic material than

usage tests and (5) having potential for standardization of tests methods (Vinken and Blaauboer, 2017). In all cytotoxicity tests, the test system itself must be nontoxic, sterile, and reproducible so as not to interfere with the analysis of the material. It should be emphasized that the cytotoxicity test alone cannot predict the overall biocompatibility of material (li et al., 2015).

Development of Tissue

Culture technique

Development of tissue culture technique is the cultivation of tissue in vitro, these cells are characterized by (Espinosa-Leal et al., 2018), growth, differentiation, and metabolism, it develops quite naturally from some of the technique of embryology, in 1903 Jolly performed experiment which marked the 1st observation on cell survival and cell division in vitro (Jolly, 1903), Alexis Carrel a noble prize-winner surgeon demonstrated without doubt that animal cell could be grown indefinitely in vitro, this was the step which leads to the development of tissue culture technique which was laid down by many Scientists (Murphy 1913, Harrison 1928, John paul 1975). The subsequent fats of these cells vary enormously, many of them are quite short-lived, like blood cells, others frequently persist in culture some time for months without dividing at all, and then eventually die, like neurons and muscle cells.

Method

1-Preparation of cell culture

The cell used in this experimental study were permanent Vero cells Growth medium for these cells consisted of Roswell park memorial medium (Yang et al., 2020) supplemented with penicillin 100000ug/ml and streptomycin 100ug/ml with 10 percent fetal bovine serum (FBS) 150ul of proliferating Vero cell were cultured in three tissue culture plates for 48 hours before starting the experiment and were incubated at 37c,100 percent humidity and 5 percent co_2. The tissue culture plate is a sterile plastic or glass plate with 96 well arranged in (8)horizontal lines each line has (12) wells.

2-Preparation of seslar extracts

Each sealer was mixed according to manufactural instruction using a sterile spatula and glass cement slab. The set sealer was allowed to set in 37c,100percent humidity for 48 hs to prepare the extract 450mlg of set sealer was put in a separated (25ml) test tube containing 10ml of PBS. The extract was withdrawn at the end of (7) days for cytotoxicity evaluation.

The same method of extract preparation was used by (Abubakar and Haque, 2020) in studying the cytotoxicity of root canal filling material. In this experimental study, an extract of sealer was used because it is easily prepared and resembles the presence of material in the living body Prosecute.

The purpose of this experimental study is to record the cytotoxic effect of four root canal sealers by adding their extract to the cultured Vero cell, the number of viable cells will be decreased due to the effect of the cytotoxic agent Only the viable cells will take the vital dye (Neutral red) The optical density of the dye can be read by using (ELISA reader) increased optical density means less cytotoxicity before adding the extract the horizontal lines were labeled according to the type of sealer one line was served as control 50ul of extracts of set sealer (Thakore and Srivastava, 2017). (Al-Shehri et al., 2017) were added to the cultured Vero cell in three tissue culture plates the tissue culture plate were put in air jar and incubated at 37c,100percent humidity, and 5 percent Co_2 at the end of each experimental time the plate was taken from the air jar for staining, this process was carried by adding 50ul of 0.01 percent neutral red (vital dye) to the cultured well. After two hours the excess dye was removed by washing the plate three times with PBS, then after 50ul of phosphate buffeted ethanol was added to each well to extract the dye from the viable cell. The amount of dye that was taken by the viable cell can be read by (an ELISA reader), which is a photo-sensative machine, designed to read the optical density of each well. increased optical density due to increase viable cells will give us higher values. The same process was carried out after48h, and 72h and all results were read by the same method.

The above method which was used to record the cytotoxicity was applied by (Garg et al., 2018) for the detection of clostridium per fringers enterotoxin.

Statistical Analysis

The cytotoxicity of medium extract of four root canal sealers was evaluated by using a Vero cell line, the number of the killed cell can be evaluated by reading the optical density of each well by the mean of (ELISA reader), and the mean was determined from five replicant in the same way the mean value for control were determined from four replicate (one control value for each plate).

Descriptive results

The arithmetic mean and standard deviation were presented in Table 1 which shows the mean values for four types of sealers in three experimental times, 24h, 48h, and 72h.

Table 1: Description statistics, including means, and standard deviation

		Watch	Endo	Dorifill	Diaket
1 day	Mean	97.4	37.2	42	51.5
	S.D	12.621	2.588	5.292	11.387
2 days	Mean	138.4	68	47.8	44.6
	S.D	2.881	4.301	1.304	6.427
3 days	Mean	260.8	272.2	233.8	233.8
	S.D	38.324	42.863	23.847	24.067

Cytotoxicity evaluation of endodontic sealer

In this respect, the statistical analysis of data showed that the difference observed between control (untreated well) and the sealers (treated well) were very highly significant at all levels.

This indicates a great reduction in the number of viable cells due to the cytotoxicity effect of sealer.

Table 2: Statistical results show the differences between types sealers and the control

Time	Material	Recent		
		t	df	p
1 day	Watch	5.612	11	***
	Endo	10.990	11	***
	Dorifill	10.496	11	***
	Diaket	8.835	11	***
2 days	Watch	9.546	11	***
	Endo	13.457	11	***
	Dorifill	14.630	11	***
	Diaket	14.701	11	***
3 days	Watch	13.098	11	***
	Endo	12.229	11	***
	Dorifill	15.544	11	***
	Diaket	15.526	11	***

Effect of time on cytotoxicity level of sealer

These experimental results were present in Table 3, These results studied the difference in cytotoxic degree between the 1st and 2nd day and between the 2nd and 3rd experimental day.

Table 3: Statistical results show the effect of time on the degree of cytotoxicity of sealer

	Material	1 day – 2 days			2 days – 3 days		
		t	df	p	t	Df	p
Recent	Watch	-7.082	8	***	-7.122	8	***
	Endo	-13.719	8	***	-10.600	8	***
	Dorifill	-2.380	8	*	-17.414	8	***
	Diaket	1.649	8	NS	-16.984	8	***

Discussion

Biocompatibility of dental material can be evaluated either in vivio or in Vetro, The in vitro experiments are simple, reproducible, inexpensive, and suitable for initial screenings of materials cytotoxicity and it also precludes the use of experimental animals, many methods have been proposed to assess in vitro the cytotoxicity of dental material.

Conclusion

The four endodontic sesles used in this experimental study show cytotoxic effect, the cytotoxic effect of all sealer was reduced by time, after three days the sealers still show high cytotoxic effect ر Rankiny of cytotoxic is different according to their composition, Enfomethason show highest cytotoxicity followed by Diket, Dorifill and Wach sealer. WE CAN APPLY THIS PROCEDURE (IN VITRO ASSESSMENT) FOR MOST MEDICAL RESEARCH INSTEAD OF ANIMAL STUDY.

References

Abubakar, A. R., & Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of pharmacy & bioallied sciences, 12(1), 1.

Abubakar, A. R., & Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of pharmacy & bioallied sciences, 12(1), 1.

Al-Shehri, S., Al-Nazhan, S., Shoukry, S., Al-Shwaimi, E., & Al-Shemmery, B. (2017). Root and canal configuration of the maxillary first molar in a Saudi subpopulation: a cone-beam computed tomography study. Saudi Endodontic Journal, 7(2), 69.

Berman, Louis H., and Kenneth M. Hargreaves. Cohen's pathways of the pulp-e-book. Elsevier Health Sciences, 2020.

Espinosa-Leal, C.A., Puente-Garza, C.A., & García-Lara, S. (2018). In vitro plant tissue culture: means for production of biological active compounds. Planta, 248(1), 1-18.

Furtado, T. C., de Bem, I. A., Machado, L. S., Pereira, J. R., Só, M. V. R., & da Rosa, R. A. (2021). Intratubular penetration of endodontic sealers depends on the fluorophore used for CLSM assessment. Microscopy research and technique, 84(2), 305-312.

Garg, S., Huifu, H., Kaul, S. C., & Wadhwa, R. (2018). Integration of conventional cell viability assays for reliable and reproducible read-outs: experimental evidence. BMC research notes, 11(1), 1-7.

Harrison RG., (1928). On the status and significance of tissue culture.Arch. exp. Zellforsch. 6,4.

Jain, S., & Adhikari, H. D. (2018). Scanning electron microscopic evaluation of marginal adaptation of AH-plus, GuttaFlow, and RealSeal at apical one-third of root canals–Part I: Dentin-sealer interface. Journal of conservative dentistry: JCD, 21(1), 85.

Jolly, J., (1903). Sur La. Duree de la Vie de la multiplication des cellules animales en dehors de l'organisme, G.R. Soc. Biol. (Paris). 55: 1266.

Kunert, M., & Lukomska-Szymanska, M. (2020). Bio-inductive materials in direct and indirect pulp capping—a review article. Materials, 13(5), 1204.7

Li, W., Zhou, J., & Xu, Y. (2015). Study of the in vitro cytotoxicity testing of medical devices. Biomedical reports, 3(5), 617-620.

Munaco FS., Miller WA., Everett MM., (1978). A study of long-term toxicity of endodontic materials with use of an in vitro model. JOE; 4:151, May. Murphy J.B., (1913). Transplantibility of tissue to the embryos of foreign species. J. exp. Med. 17, 482. Murphy WM., (1988). The testing of endodontic material in vitro. Int.Endodon. J. 21: 170-7.

Puck, T.T., (1964). Studies of the life cycle of mammalian cells. Cold spr. Harb. Symp. Quant. Biol. 29: 167. Cited by John Paul, cell and tissue culture, 5th ed. Chapter 3. Behavior of cell in culture, growth, differentiation and metabolism

Root and canal configuration of the maxillary first molar in a Saudi subpopulation: A cone-beam computed tomography study

Shashirekha, G., Jena, A., Pattanaik, S., & Rath, J. (2018). Assessment of pain and dissolution of apically extruded sealers and their effect on the

periradicular tissues. Journal of conservative dentistry: JCD, 21(5), 546.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6161529/

Tabassum, S., & Khan, F. R. (2016). Failure of endodontic treatment: The usual suspects. European journal of dentistry, 10(01), 144-147.

Tan, K. S., Yu, V. S. H., Quah, S. Y., & Bergenholtz, G. (2015). Rapid method for the detection of root canal bacteria in endodontic therapy. Journal of endodontics, 41(4), 447-450.

Thakore, D., & Srivastava, A. K. (2017). Production of biopesticide azadirachtin using plant cell and hairy root cultures. Engineering in Life Sciences, 17(9), 997-1005.

Vinken, M., & Blaauboer, B. J. (2017). In vitro testing of basal cytotoxicity: establishment of an adverse outcome pathway from chemical insult to cell death. Toxicology in vitro, 39, 104-110.

Yang, L., Fan, X., Zhang, J., & Ju, J. (2020). Preparation and characterization of thermoresponsive Poly (N-Isopropylacrylamide) for cell culture applications. Polymers, 12(2), 389.