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Comparative Study of Modified Chlorhexidine Toxicity, Chlorhexidine and Sodium Hypochlorite on Gingival Fibroblast Cells L929 *In Vitro*

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ABSTRACT

One obvious and good properties of sodium hypochlorite is capacity of tissue solubility which has effective role in direct root canal cleaning, necrotic tissues and root canal system complexity. Chlorhexidine, in spite of all advantages that have than sodium hypochlorite, lacks this property. Superficial active factors or surfactants are materials that are used extensively as irritant, emulsifier, disinfection and solution additive. Surfactants are different types including benzalkonium chloride 4% and sodium lauryl Sulfate 2%. In this research, sodium dodecyl sulfate was used. Gingival fibroblast cells are kept in 25 cm² flasks in 37⁰C temperature. And then they are cultivated in Gibco Dulbecco's Modified Eagle Medium (DMEM). 96 wells plates with 50 microliter are filled with growth medium with 2000 fibroblast and it is placed in incubator for 24 hours. After 24 hours, growth medium is thrown away and all plates are washed with phosphate buffered saline (PBS). All experimental steps are performed for preventing samples being contaminated under hood. The experiment was carried out as triplicate (3 wells cell). Results: In order to perform a research, Tukey-test and Duncan test were applied for explaining research variable. Results: toxicity rate of under tested solutions on fibroblast cells for group 1-4 are respectively (right to left) 13.75, 51.75, 20.75 and 51.75. Results of comparing means in four groups in landa level 4 indicated that groups are not significantly different in toxicity rate. results of this research indicated that this mixture can be used as an appropriate candidate for replacing sodium hypochlorite based on its disadvantages which needs to be studied more.

Key words: Sodium Hypochlorite**,** Chlorhexidine, Modified Chlorhexidine, *Fibroblast* Cells, Toxicity Rate.

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INTRODUCTION

If cleaning is not carried out appropriately, necrotic soft tissue remnants are acting as nutrition source for remained bacteria and can cause canal pollution [1-3]. Thus, main aim in endodontic treatment is eliminating microorganism from root canal and providing an environment for periapical tissue repairing [1,4,5, 6-14]. So root canal space does not become as a source for infection [15].

This goal is met by eliminating diseased tissues and preventing second pollution [16-18]. A dentist needs to have sufficient information and expertise for this job [9]. Mechanical use of tools cannot clean canal convoluted tubule network [1, 5, 13, 19]. With these methods, preparing 40-50% canal

wall is remained intact and so sufficient infectious tissue is remained so microorganism can be alive and grow again [15]. Accordingly, using irrigant besides mechanical preparation is needed [1, 2, 5, 7]. In addition to irrigant which are not anti-microbial, using mechanical tools can reduce 50% of bacteria in root canal [20].

Using appropriate irrigants in non-surgical root treatment has been ignored in dentistry students and clinical issues' education [5]. Disinfecting root canal system by preparation and using irrigants has key role in reducing bacteria numbers from root canal and helping to control periapical disease [9]. This material completes mechanical debriman by flushing out debris, solving necrosis tissues, removing smear layer and disinfecting root canal system [10, 17, 21]. No unique solution can apply for this all [17].

Studies indicated that regular methods of using tools in cleaning and shaping produce smear later which covers canal walls and dentinal tubule neteris [22-24]. This layer's diameter is 1-2 mμ and it is amorphous and disordered [11] and it is made from pulp as organic material, inorganic dentinal debris, microorganisms, their products and necrotic material [11,14,22,23,24]. Smear layer prevents drug penetration into canal to root canal system and dentinal tubules and in addition it prevents compatibility of filling material with surface of prepared canal walls [23, 24].

Giannelli M.1 *et al* in 2008 in Italy studied chlorhexidine effect on osteoblast, fibroblast and endothelial. Results indicated that CHX indicated its toxic effect on cells in special time and dependent to the doze [1].

De Souza LB *et al* in 2007 in Sao Pauloin studied effect of different densities of chlorhexidine and peroxide hydrogen on odontoblast-like cells. Results indicated that CHX 0.02% has high toxicity effect on odontoblast-like cells while chlorhexidine 0.004% and 0.0024% had toxic effect on cells [25].

Barnhart.3 *et al* in 2005 in US performed a study on toxicity effect of sodium hypochlorite, potassium iodide, calcium hydroxide and chlorine dioxide on fibroblast cells. Results indicated that IKI and $Ca(OH)_2$ had low toxicity that SCD, NAOCLl and betadine. LKL and $Ca(OH)_2$ are tolerated by fibroblast cells simply [23].

Yu-chao chang *et al* in 2001 in America studied chlorhexidine and sodium hypochlorite effect on periodontal ligament. Results indicated that sodium hypochlorite and chlorhexidine has toxic effect on cells while CHX inhibit protein synthesis and sodium hypochlorite had no effect. In addition, CHX and sodium hypochlorite had inhibitory effects on mitochondria [26].

In this study, in order to compare toxicity of modified chlorhexidine, chlorhexidine and sodium hypochlorite on gingival fibroblast cells L929 in vitro, toxicity rate of NAOCL(2.5% and 5.25%), chlorhexidine and modified chlorhexidine on fibroblast cell in different densities are determined and their toxicity rate is compared.

MATERIAL AND METHODS

Materials and required tools

Applied tools are fibroblast cellular live, growth medium, 96 wells plates, LDH Plus kit, phyisiologic serume, blade agar growth medium, TSB growth medium, sodium thiosulfate, Tween-8, lecithin, coloride barium, sulfuric acid, sodium hypochlorite, chlorhexidine 95%, modified chlorhexidine, petridish, loop culture, cell culture plate and faucet with incubator, incubator OC37, digital scale, autoclave, spectrophotometry, sonicatore machine, optical microscope, heater stirrer, vortex, sampler, Elisa Reader.

Methodology

Gingival fibroblast cells in third or fourth passage which is provided from cell bank of Tehran Pasteur Institute are used. They are kept in 25 cm^2 flasks in 37^0 degrees and they were cultivated in Gibco Dulbecco's Modified Eagle Medium (DMEM) which includes 1% Fetal Calf Serum (FBS), penicillin, 100000 mg Streptomycin, 50 mg gentamicin and 125 mg Fungi zone. Flasks are placed in incubator in 37^0 c with 5% carbon dioxide and 95% air. Growth medium is changed every other day until maximum growth of fibroblast cells and then it is separated by 0.25% EDTA trypsin and then it is counted by hemocytometer. 96 wells plate is filled with 50 microliter growth medium with 20000 and it is located in incubator for 24 hours. After 24 hours, growth medium is thrown away and all plates are washed with phosphate buffered saline. All test steps is performed under hood in order to prevent sample pollution. The test is performed triplicate (3 wells cell) [1].

Test group are as follow:
Group 1: sodium hypochlorite 5.25%
Group 2: sodium hypochlorite 2.5% obtained from diluting sodium hypochlorite 5.25% with normal saline.
Group 3: chlorhexidine 0.2%
Group 4: modified chlorhexidine which is obtained from mixing sodium dodecyl sulfate and 0.2% CHX.

In this test, cell toxicity rate is formed via formation from tetrazolium is affected by lactate dehydrogenase (LDH) which is cell Cytoplasm and mitochondrial succinate dehydrogenase and it is active only in live cells so it is measured by determining absorption value with ElISA method and by ELISA reader with wave length equals to 490, 492 nanometers respectively (LDH Plus kit with serial number as 11644793001 made in Roche of German).

In order to measure toxicity via LDH, 3 other groups including high control background and low control are needed in addition to experimental groups. So that in background group, growth medium, in high control group, the lubricant which is in kit (white cap) are used for liberating LDH and lubricating cells, and they are placed in low control group. In experimental groups, after 15 minutes each irrigants near to cells in incubator, 100 microliter from reaction mixture are added to cells. And then for 30 minutes they are placed in incubator; then 50 microliter of stop solution is added and after 1- seconds shaking, absorption rate is measured.

Statistical method
In this study, dependent variable is toxicity of canal irrigant which is measured based on turbidity rate and its scale is rational. In addition, independent variable is an irrigant that it is used according to methodology and commercial name. Scale of this variables sodium hypochlorite 2.5% and 5.52% and chlorhexidine 0.2 and 1 and 2% and modified chlorhexidine.

Based on last researches, sample volume is based on 3 repetitions [1]. For carrying out this research we used LSD and ANOVA (Density and different materials) and descriptive statistic table is used for explaining variables.

RESULTS

Cell toxicity mean of under tested data is as table 1.

Results of LSD test are indicated in table 2. This test is performed for comparing different groups statistically and when significance value is less than 0.05, results of the study is significant.
As it is clear in table 2, tested material in density of 2 and 4 landa (group 7 and 8) has significant difference rather that sodium hypochlorite 2.5% and chlorhexidine in density of 2 landa and tather than sodium hypochlorite 2.4% and chlorhexidine in both densities of 4 landa. Other materials have significant toxicity in some densities. As these materials are not target of this study we did not explain them. Toxicity diagram is as Table 2.

RESULTS AND DISCUSSION

In this study toxicity of modified chlorhexidine, chlorhexidine and sodium hypochlorite on gingival fibroblast cells L929 in vitro was compared. And toxicity rate of NAOCL (2.5% and 5.25%), chlorhexidine and modified chlorhexidine on fibroblast cells in different densities were determined. Results of toxicity resulted from modified chlorhexidine is not comparable with results of other articles because it is completely new material and it needs to be more investigated.

Table 1: Cell toxicity mean of different groups

Groups		N	Mean	Std. Deviation	Minimum	Maximum
Hipo 5.25% in 2 landa scale	1.00	4	38.2500	38.43067	4.00	93.00
Hipo 5.25% in 4landa scale	2.00	4	49.5000	34.54948	4.00	81.00
Hipo 2.5% in 2 landa scale	3.00	4	66.2500	12.52664	52.00	81.00
Hipo 2.5% in 4 landa scale	4.00	4	22.7500	19.75475	4.00	50.00
Col 0.2% in 2 landa scale	5.00	4	65.5000	7.76745	57.00	75.00
Col 0.2% in 4 landa scale	6.00	4	53.5000	21.14237	25.00	71.00
Modified chlorhexidine in 2 landa scale	7.00	4	27.6250	12.72383	12.50	42.00
Modified chlorhexidine in 4 landa scale	8.00	4	18.0000	11.63329	4.00	31.00
	Total	32	42.6719	26.66205	4.00	93.00

Table 2: LSD test

(I) g8	(J) g8	Mean Difference (I-J)	Sig.
1.00	2.00	-11.25	.485
	3.00	-28.00	.090
	4.00	15.50	.338
	5.00	-27.25	.099
	6.00	-15.25	.346
	7.00	10.63	.509
	8.00	20.25	.214

(a): LSD Test 1

(I) g8	(J) g8	Mean Difference (I-J)	Sig.
2.00	1.00	11.25	.485
	3.00	-16.75	.301
	4.00	26.75	.105
	5.00	-16.00	.323
	6.00	-4.00	.803
	7.00	21.87	.180
	8.00	31.50	.058

(b): LSD Test 2

(I) g8	(J) g8	Mean Difference (I-J)	Sig.
3.00	1.00	28.00	.090
	2.00	16.75	.301
	4.00	43.50*	.011
	5.00	0.75	.963
	6.00	12.75	.429
	7.00	38.62*	.023
	8.00	48.25*	.006

(c): LSD Test 3

(I) g8	(J) g8	Mean Difference (I-J)	Sig.
4.00	1.00	-15.50	.338
	2.00	-26.75	.105
	3.00	-43.50*	.011
	5.00	-42.75*	.013
	6.00	-30.75	.064
	7.00	-4.875	.761
	8.00	4.75	.767

(d): LSD Test 4

(I) g8	(J) g8	Mean Difference (I-J)	Sig.
5.00	1.00	27.25	.099
	2.00	16.00	.323
	3.00	-0.750	.963
	4.00	42.75*	.013
	6.00	12.00	.457
	7.00	37.87*	.025
	8.00	47.50*	.006

(e): LSD Test 5

(I) g8	(J) g8	Mean Difference (I-J)	Sig.
6.00	1.00	15.25	.346
	2.00	4.00	.803
	3.00	-12.75	.429
	4.00	30.75	.064
	5.00	-12.00	.457
	7.00	25.87	.116
	8.00	35.50*	.035

(f): LSD Test 6

(I) g8	(J) g8	Mean Difference (I-J)	Sig.
7.00	1.00	-10.62	.509
	2.00	-21.87	.180
	3.00	-38.62*	.023
	4.00	4.87	.761
	5.00	-37.87*	.025
	6.00	-25.87	.116
	8.00	9.62	.550

(g): LSD Test 7

(I) g8	(J) g8	Mean Difference (I-J)	Sig.
8.00	1.00	-20.25	.214
	2.00	-31.50	.058
	3.00	-48.25*	.006
	4.00	-4.75	.767
	5.00	-47.50*	.006
	6.00	-35.50*	.035
	7.00	-9.62	.550

(h): LSD Test 8

** The mean difference is significant at the 0.05 level.*

However, in Barnhart *et al* study it was identified that IKI and $Ca(OH)_2$ have less toxicity than NAOCL, SCD and betadine. LKL and $Ca(OH)_2$ are tolerated by fibroblast cells easily [27]. More rate of sodium hypochlorite toxicity is compatible with this study.

In addition, results of Yu-chao chan *et al* experiment indicated that sodium hypochlorite and chlorhexidine has toxic effect on the cells. But CHX inhibited protein synthesis and sodium hypochlorite did not have this effect. In addition, CHX and sodium hypochlorite had inhibitory effect on mitochondria activity [28]. Chlorhexidine and sodium hypochlorite toxicity was compatible with our results although applied cell is different with this study.

CONCLUSION

As it is indicated, it can be concluded that common irrigants such as sodium hypochlorite and chlorhexidine have toxic effect on biologic cells. Rate of modified chlorhexidine toxicity had no significant difference with 3 other products of the experiment including sodium hypochlorite 5.25%, sodium hypochlorite 2.5% and chlorhexidine 2.5%. So it can be concluded that this material is not more toxic than other endodontic irrigants.

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