Cytotoxic Effect of Modified Gutta Percha by Incorporating Bioactive Glass 45S5 and Chitosan Particles As Potential Root Canal Filling Material

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Abstract

Aim: To investigate the cytotoxic effects of modified bioactive and antimicrobial gutta percha on the fibroblast cells.

Methods: The cytotoxic effects of modified bioactive and antimicrobial gutta percha on the fibroblast cells were investigated using Methylthiazol tetrazolium (MTT) assays at different times 24, 48 and 72 hours.

Results: there is no significant difference between controle and new modified gutta percha at first 24 hrs. while with time at 48 and 72 hrs. there were significant difference.

Conclusion: Bioactive bioglass BG45S5 and chitosan showed *in vitro* cytotoxic effects when mixing with gutta percha in fibroblast cells as demonstrated by MTT assays, although toxic effect was observed mostly as the same to control gutta percha.

Keywords: *Gutta percha, bioactive bioglass BG45S5, chitosan, MTT assay, fibroblast cells, cytotoxic effect.*

Introduction

Root canal treatment is one of the techniques practiced most commonly in dentistry to save the tooth and keep it in a functional position in the oral cavity. The final filling of RCT is called obturation and biocompatible material used to fill the root canal is called an obturating material [(11)]. Their basic function is to fill the root canal and seal the apical foramen. The basic objectives of root canal obturating materials according to Noort, are to provide a clean canal, free of bacteria and other debris, provide an apical seal to prevent the fluids from tissues to enter into the canal, irritants leaving the canal and to prevent recontamination due to oral micro-organisms [(23)]**.** Since its introduction in dentistry, gutta-percha has been the most widely used solid-core root canal filling material [(9,20)]**.** demonstrated that gutta-percha appeared to be the least toxic material in studies in vitro. They also pointed out that composition of gutta-percha points for root canal filling may vary according to the manufacturer and that each brand should be

evaluated separately for its toxicity. Considering that the real composition of gutta-percha mixtures is not provided by any manufacturer, it can also be assumed that it may vary with batches. The commercially available materials are although biocompatible but not bioactive. One of the recent trends in endodontics has been the development of obturating materials that are capable of bonding to canal wall dentin to eliminate interfacial gaps coronally and apically [10,9]. Dentin adhesive technology has been adapted from restorative dentistry and applied to obturating materials through hybrid layer formation [12].

With an era of biomimetic dentistry, Bioactive materials proved useful biological healing and beneficial for the tissues. Bioactive materials can define as "a compound when implanted into the body does not produce any injurious effects and also has the ability of eliciting a response from living tissue, organisms or cell such as inducing the formation of hydroxyapatite.". For a material to be Bioactive it must be bactericidal, bacteriostatic, sterile, stimulate reparative dentine formation, and maintain pulp vitality. A bioactive material consists of bioactive calcium phosphate ceramics, bioactive glass ceramics and bioactive composite [8]. The interaction between bioglass (as biomaterial) and simulated body fluid is important in order to predict the apatite surface layer formation, which is able to chemically interact with bone tissue [4]. Likewise, bioactive glasses of the SiO2-Na2O-CaO-P2O5 system have been shown to possess antimicrobial activity through the release of ionic alkaline species. These can be used as dentine disinfectants to offer an alternative to calcium hydroxide [13].

Chitosan, a natural and non-toxic polymer, is produced by the deacetylation of chitin and has received considerable attention in a wide range of applications due to their biological (anti-microbial, bio-adhesive, bio-compatible, and binding agent) properties. These advantages and applications of chitosan suggest its potential usage in root canal treatment and some authors have already carried out some preliminary assays in this field [5].

Methodology

A- Fabrication of new bioactive gutta percha:

The total amount of the Gutta Percha (Dentsply Maillefer, Switzerland) was weighed by four digits sensitive balance ADAM AFA-210LC (UK) which was 1.5 gm. The filer weights were 1% for bioactive powder 45S5 (MO-SCI, USA) and chitosan (SHAANXI SANGHERB BIO-TECH INC, China) where they incorporated by replacement of 0.015 gms (for each filer) of the Gutta-Percha with same weights of powder. So, these percentages were clinically applicable (ISO 6876/2012 standards).

Fabrication of Gutta Percha

Gutta percha points (1.5 gms) were taken and placed in glass beaker. The beaker has been placed in electrical oven CARBOLITE (UK) at 200ºC for 15min. the beaker has been taken out of the oven, then the gutta percha points became semi-soft. A small chloroform (Riedel-de Haën, German) amount (5ml) has been added to the beaker to solve the gutta percha with continues moving of solvent with glass stick till complete solvent of gutta percha and become like suspension of liquid [11].

Preparing of filers

The bioactive bioglass (with weights 0.015gms represent filler percentage 1%) was dissolved in formic acid (SCR-China) (5ml) and stirring for 3 days until all particles was dissolved. Then the chitosan (with weights 0.015gms represent filler percentage 1%) has been dissolved in 1.0% of the acetic acid (MERCK- German) (v/v) by using magnetic stirrer.

The viscous chitosan was adding to previous viscous bioactive bioglass and mix by using a magnetic stirrer. All the viscous mixture of bioactive bioglass and chitosan was adding and mixed together and the total fillers weight was 0.03gm.

Mixing the gutta percha with filers

All the mixture of filler was adding slowly to solvent gutta percha and mixed with a magnetic stirrer till the materials acquired a semi viscous state, it placed in glass preti dishes until complete drying and setting.

Preparing of sample as a disk

A mold was design and fabricated to acquired materials to produce a 5mm width and 2mm height of materials. A 0.035gms of materials was selected and add to cylinder of fabricated devices and a constant scrowing for 1 min was applied to materials to obtained a homogenous disc for all groups.

Fig (1-1) Special mod ready to compress the mixture.

Fig (1-2) Special mod parts.

Fig (1-3) disc of gutta percha (0.035 grms) with 5mm width and 2mm height.

Fig (1-3) disc of gutta percha (0.035 grms) with 5mm width and 2mm height.

B- Cytotoxicity study of new bioactive gutta percha

Cell Line (Human Dermal Fibroblast, neonatal) & Cell culture

Those cells underwent a small number of the population doublings, which is why, they are more representative of main functional tissue component from which they have been obtained, compared with the continuous (tumors or artificially immortalized) cell lines, which makes the primary cells into more representative model for in vivo states [24]. Cells were cultured in Dulbecco's Modified Eagle medium (Life Technologies, Inc., Rockville, MD, USA) supplemented with heat-inactivated fetal bovine serum (10%; Sigma-Aldrich, St. Louis, MO, USA), streptomycin, penicillin (1%), and glutamine (2 mmol/L).

Biological activity

Methylthiazol tetrazolium (MTT) assays were carried out at the Natural Product Research and Drug Discovery Centre, Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.

Cytotoxicity assay (MTT assay)

MTT (3-(4,5-dimethylthiazol-2-yl)-2–5-diphenyltetrazolium bromide) assay is one of the most commonly used colorimeteric assay to assess cytotoxicity or cell viability [1].

The MTT assay was carried out in the fibroblast cell line to determine the cytotoxicity activity of new bioactive gutta percha. New bioactive gutta percha was dissolved in dimethyl sulfoxide (DMSO) to produce a stock solution and serial dilutions were prepared (0.78125-200 μg/mL). Control gutta percha, were added to fibroblast cells and the cell cultures were incubated for 24,48 and 72 hrs in a CO_2 incubator. MTT (5 μg/mL) was added to each well and the plates were incubated further for 1-4 h. The media was removed and DMSO was added to each well to solubilize the formazan crystals. The absorbance was measured by the use of a Hidex Chameleon microplate reader (LabLogic Systems Ltd., Sheffield, United Kingdom) at 575 nm.

Data

Data were collected using Excel (Microsoft Office 2010, Microsoft Corp., Redmond, WA). SPSS software (IBM Software, version 22) was used to analyze the data.

MTT results

The dose-response (mean and standard deviation) of fibroblast cells treated with new bioactive gutta percha was illustrated in table (1-1).

MTT (3-(4,5-dimethylthiazol-2-yl)-2–5-diphenyltetrazolium bromide) assay is one of the most commonly used colorimeteric assay to assess cytotoxicity or cell viability [1]. This assay determines principally cell viability through determination of mitochondrial function of cells by measuring activity of mitochondrial enzymes such as succinate dehydrogenase [21].

In this assay, MTT is reduced to a purple formazan by NADH. This product can be quantified by light absorbance at a specific wavelength. Return to our results, there is no significant difference between controle and new modified gutta percha at first 24 hrs. while with time at 48 and 72 hrs. there were significant difference.

Table (1-1) Means and standard deviation for new gutta-percha.

Row state		Control			New bioactive gutta percha		
		Mean	SD	No.	Mean	SD	No.
1	24	84.799	1.238	3	81.790	1.447	3
2	48	78.318	2.892	3	73.302	1.580	3
3	72	73.765	1.238	3	69.445	1.104	3

Table (1-2) A one-way ANOVA test for new gutta-percha at different time value.

Sidak's multiple comparisons test	Significant?	Summary	Adjusted P Value
Control - sample			
24	No	ns	0.1437
48	Yes	-	0.0104
72	Yes	-	0.0262

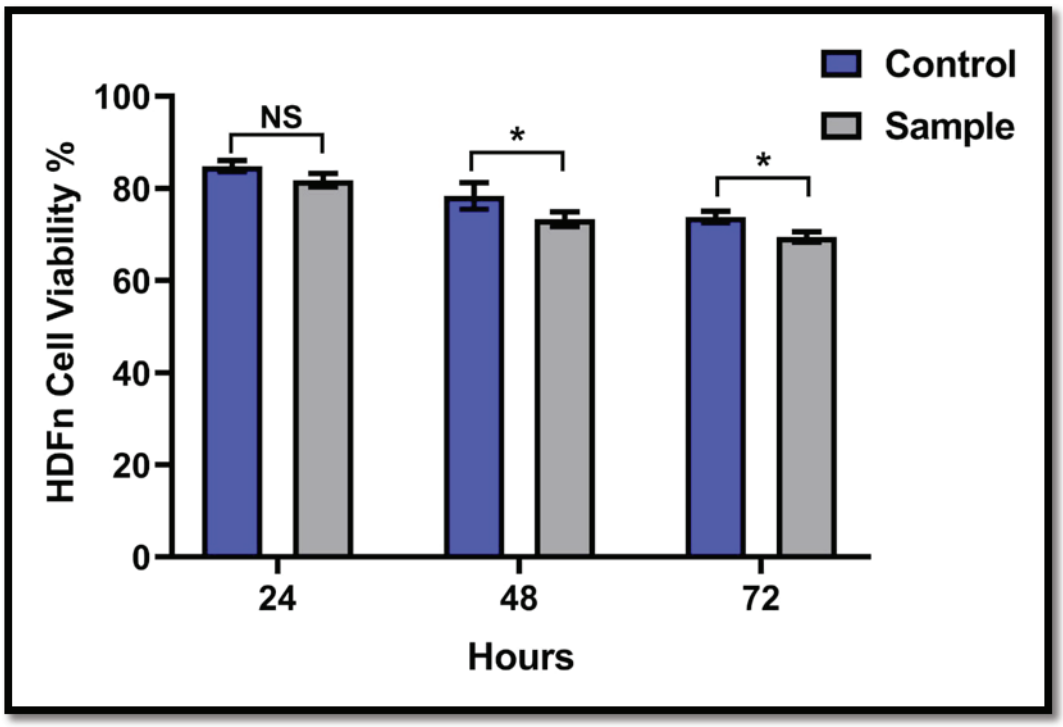

Fig. (1- 4) MTT assay bar-chart for new gutta-percha in different time intervals.

Discussion

The gutta-percha inertness has been challenged via [17] showing that the material might have high toxicity in specific tissue culture assays. A study conducted by [16] **(A&B)** indicated that the cones of gutta-percha have fairly slow acting, weak, yet considerable inherent antimicrobial property that is due to the zinc oxide component. A study conducted by [6] indicated that the human pulp fibroblasts remains round and were failing in attaching and proliferating around cut gutta-percha points.

The dentinal tubules allow diffusion of materials placed into the root canal system. Furthermore, contact of these materials with periodontal tissues may occur via the apical foramen or accessory root canals. In this context the toxicity of a substance, i.e. its biocompatibility in contact with surrounding tissues, plays an important role. Primary toxicity can be investigated by means of gingival fibroblasts as so-called "target cells," whereas the reaction of in vivo tissue depends on further parameters such as salivary flow rate and resident bacterial flora, mechanical, and chemical stimuli during food intake, hormonal status, and this agree with [22].

In our study, the results showed that the experimental gutta percha with no significant difference at 24 hrs. with control gutta percha, this may be due to no low concentration of bioactive glass and chitosan as fillers, and the zinc oxide was the prominent material at first time. This was agreement with [18] as high content related to zinc oxide in the formulations of gutta-percha explaining the toxicity regarding gutta percha points. Also, our results agree with [14] how concluded the adding cytotoxicity is increased via adding a glass to gutta percha points. After period of time; the experimental gutta percha have significant difference with control gutta percha; this due to fact that the bioactive glass and chitosan have bactericidal properties and when mixed with gutta percha it showed more toxicity (increase in the number of dead cells), this agreement with [3]. Another fact may be due to the activity of fillers, cause the ionic release anticipated to enhance the material's bioactivity leading to the cytotoxicity to be increased. This agree with [14].

Conclusion

Bioactive bioglass BG45S5 and chitosan showed *in vitro* cytotoxic effects when mixing with gutta percha in fibroblast cells as demonstrated by MTT assays, although toxic effect was observed mostly as the same to control gutta percha.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None:

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