

Research Report

Biocompatibility of acrylic resin after being soaked in sodium hypochlorite

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ABSTRACT

Background: Acrylic resin as basic material for denture will stay on oral mucosa for a very long time. The polymerization of acrylic resin can be performed by conventional method and microwave, both produce different residual monomer at different toxicity. Acrylic resin can absorb solution, porous and possibly absorb disinfectant as well, that may have toxic reaction with the tissue. Sodium Hypochlorite as removable denture disinfectant can be expected to be biocompatible to human body. The problem is how biocompatible acrylic resin which has been processed by conventional method and microwave method after being soaked in sodium hypochlorite solution. **Purpose:** The aim of this study was to understand in vitro biocompatibility of acrylic resin which has polymerized by conventional method and microwave after being soaked in sodium hypochlorite using tissue culture. **Methods:** Four groups of acrylic resin plate were produced, the first group was acrylic resin plate with microwave polymerization and soaked in sodium hypochlorite, the second group was acrylic resin plate with microwave polymerization but not soaked, the third was one with conventional method and soaked and the last group was one with conventional method but not soaked, and in 1 control group. Each group consists of 7 plates. Biocompatibility test was performed in-vitro on each material using fibroblast tissue culture (BHK-21 cell-line). **Result:** The percentage between living cells and dead cells from materials which was given acrylic plate was counted. The data was analyzed statistically with T test. **Conclusion:** The average value of living cells is higher in acrylic resin polymerization using microwave method compared to conventional method, in both soaked and non soaked (by sodium hypochlorite) group. This means that sodium hypochlorite 0.5% was biocompatible to the mouth mucosa as removable denture disinfectant for 10 minutes soaking and washing afterwards.

Key words: biocompatibility, cell culture, disinfectant, acrylic resin, polymerization

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INTRODUCTION

Biocompatibility is a harmonious condition without any toxic effect on biological function, which is measured according to local cytotoxicity, systematical response, and carcinogen characteristic.¹

The treatment of a denture is by taking the denture off and soaking it at night, beside the reservation and cleansing action. The cleansing method of denture generally can be done in two ways, either mechanically or chemically. The process of chemical cleansing is done by putting the denture on the cleanser which contains disinfectant.²

In dentistry, disinfectant material play an important role to decontaminate the disposable or reusable tools

which are being used. Disinfectant materials can also be used as cleanser for acrylic resin denture. One of the disinfectant materials is sodium hypochlorite (NaOCl). As a decontamination material, the use of NaOCl is to prevent infection from patient to the medical health personnel or to other patients, especially in this era in which the number of infection caused by virus is increasing, such as hepatitis and HIV.

Sodium hypochlorite which is a chemical based material consists of chlorine, is a high level disinfectant and very effective for all bacteria, virus, yeast, parasite and spora.³ Disinfectant is aimed to prevent cross-contamination that occurs between denture's user who suffered from infection, which involved the dentist, dental technician and the

surrounding people. In daily use, denture's user can also use denture cleanser with disinfectant by soaking the denture, and rinse it with water, and put it back on. Since most part of a denture is acrylic resin which has characteristics of absorbing liquid material,⁴ it is feared that the cleansing material will eventually make in contact with the oral mucosa. NaOCl as disinfectant is toxic, especially in high concentration.⁴ It is necessary to do toxicity test of acrylic resin by using fibroblast tissue culture (BHK-21 cell-line) after being soaked in NaOCl, which is being used as disinfectant material on removable denture acrylic resin.

Beside the acrylic resin polymerization process which has been commonly used in Indonesia, microwave polymerization process becomes the latest and more efficient process which is more hygienic and resulting a better physical characteristic of acrylic resin with less residual monomer. It is important to do toxicity test of acrylic resin which polymerized by conventional and microwave method which is being soaked in NaOCl solution.

The objective of this study was to observe the in vitro biocompatibility of acrylic resin material which was polymerized by the conventional method and microwave method after being soaked in sodium hypochlorite as denture cleanser material by using tissue culture. The result of this study can give information for dentist in choosing acrylic resin denture disinfectant materials and polymerization method which is more biocompatible, so it can be guaranteed that the material which is being used is safe and more biocompatible to the oral mucosa.

MATERIALS AND METHODS

This study was done by using laboratory experimental method. The samples were twenty eight round shape plates of acrylic resin of heat cured type QC-20 (with diameter of 12 mm, and thickness of 1 mm),⁵ which were divided into four groups, with 7 plates in each group. This research was conducted at Faculty of Dentistry Laboratory of Airlangga University and Surabaya Pusvetma Laboratory.

To make the sample, mixtures of gypsum and water, was prepared with a ratio based on the manufacture's dosage, which was put into the low cuvet. Master model was put on the mixtures's surface. Each of it was 10 pieces in one cuvet, and then let it stay for 15 minutes. After the gypsum has been set, apply all the surface of gypsum with vaseline, put the top cuvet, and filled it with gypsum. The gypsum is idled until it harden. The cuvet was opened, and the master model was taken out. For conventional method of polymerization, the brass cuvet was being used, and the plastic cuvet was used for polymerization by microwave.

The mold which has been formed was filled with acrylic resin with w/p ratio according to manufacture's dosage. The cuvet was closed and then pressed it by using hydraulic bench with the pressure of 22 kg/cm² Hg.⁶

Polymerization by conventional method was performed with Japanese Industrial Standard (JIS) procedure, where

the cuvet was placed into the pan with the temperature of 70° C for 90 minutes, and then continued for 30 minutes in the temperature of 100° C, the cuvet was opened and let it cool down for 24 hours, and taken out afterward.⁷ For polymerization by microwave method, special cuvet was placed right in the centre of turn table microwave oven. Polymerization by microwave was very short. It only took 5 minutes in 500 watt microwave. The cuvet was opened when it's already cold and the plate was taken out.⁸

Nodules and excess of acrylic on the plate was cut by straight hand piece, and then were rubbed with abrasive paper, number 300 and 600, under flowing water. Heat-cured acrylic resin plate which was processed by conventional and microwave polymerization method, was round shape with a diameter of 12 mm, and thickness of 1 mm.

All plates are soaked in aquadest for 48 hours. Then the two groups were soaked in NaOCl 0.5% for 10 minutes, and the two other groups was not soaked in NaOCl solution. Concentration of NaOCl 5.25% being diluted to 0.5% (1:10), was used for soaking. The first group was plate acrylic resin which was processed by conventional method. The second group was processed by conventional method and being soaked in sodium hypochlorite 0.5% for 10 minutes. The third group was processed by the micro wave. The fourth group was processed by the microwave and being soaked in NaOCl 0.5% for 10 minutes. The fifth group was a control group, Petri dish containing media and cell culture of BHK-21, without acrylic resin plate which NaOCl 0.5 % was used as soaking material.

Acrylic resin plates were rinsed 3 times with aquadest, and sterilized with UV Lights for 1 hour inside laminar flow. Then the plate was stuck on the base of small Petri dish with silicone grease, each of one plate on one petri dish. In every petri dish was added by a media, which was incubated for 48 hours, at 37° C, CO₂ 5%. For the control group, in seven petri dish as which only contained of media without any acrylic resin plate.

In the roux tube, cell culture line BHK-21 was added with 20ml eagle media serum (EMS) 10%, and then being incubated for 48 hours at 37° C, CO₂ 5%. Cell line was tested under the microscope, and when it had been already full (*confluent*), the EMS solution was removed, and was then rinsed twice, with PBS 10%. Adding 1 ml of versin trypsin after the cell was detached, and then adding media culture which had bovine serum 10% to stop the versin trypsin reaction, and then making cell with the density of 2×10^5 . The cell was ready for the test (Figure 1).

The media solution was removed and rinsed it twice with PBS 10%, 1ml of versin trypsin 0.25%, 2 ml of EMS 10%. 0.1 ml of cell was added 0.9 ml of trypan blue, and mixed until homogenized. It was dropped on hemocytometer, the number of living cells and dead cells was calculated under the microscope, using the Bird and Forrester method (Figure 2).⁹

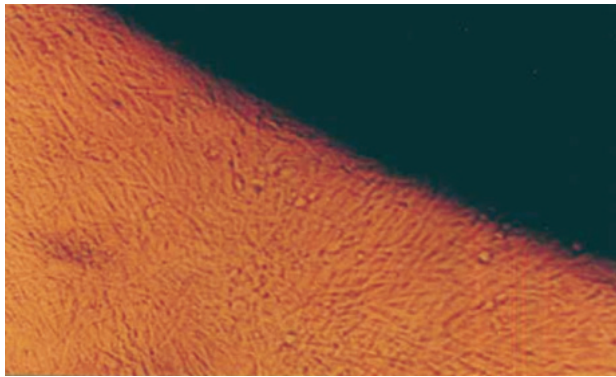


Figure 1. Microscopic appearance of BHK-21 cells which was magnified 200X, the cells is forming a firm tie and creating monolayer cell around the sample.

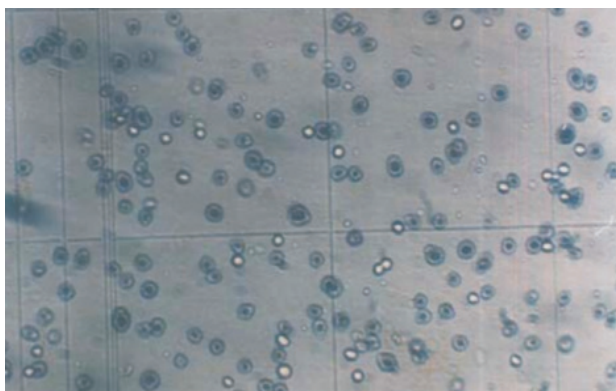


Figure 2. Microscopic appearance of the living cells (white color) and the dead cells (blue color which has absorbing trypan blue color).

RESULTS

Table 1. Means and deviation standard of living cell percentage in toxicity test of Acrylic resin which was soaked and not soaked in NaOCl to tissue culture

	Number of sample	Living Cells Percentage	
		Means	Deviation Standard
K	7	96.45	0.73
K-N	7	95.43	0.72
M	7	97.86	0.11
M-N	7	96.48	0.52
control group	7	98.04	0.20

Note:

- K : Acrylic resin processed by conventional method
 K-N : Acrylic resin processed by conventional method and soaked in NaOCl
 M : Acrylic resin processed by microwave
 M-N : Acrylic resin processed/by microwave and soaked in NaOCl

The highest mean and standard deviation of living cell percentage in toxicity test of acrylic resin which was processed by microwave, which was not soaked in NaOCl (97.86 ± 0.11) compared to the one which was

not soaked in NaOCl (96.48 ± 0.52). And for acrylic resin with conventional method which was not soaked in NaOCl (96.45 ± 0.73) is higher than acrylic resin which was not soaked in NaOCl (95.43 ± 0.72) (Table 1). To observe the level of significance of different means and deviation standard of living cell percentage in toxicity test of acrylic resin which was processed by conventional either microwave method after soaked in NaOCl, the data then was analyzed statistically by t-test (Table 2).

Table 2. The result of t test of living cell percentage of acrylic resin which is processed by conventional and microwave method and being soaked and not soaked in NaOCl

	K	K-N	M	M-N	control group
K	-	0.022 *	0.002 *		0.000 *
K-N		-		0.009 *	0.001 *
M			-	0.001 *	0.064
M-N				-	0.001 *
Control Group					

Note:

- K : Acrylic resin processed by conventional method
 K-N : Acrylic resin processed by conventional method and soaked in NaOCl
 M : Acrylic resin processed by microwave
 M-N : Acrylic resin processed /by microwave and soaked in NaOCl

T-test was done to observe the toxicity of dental material being used to the tissue culture. From the calculation of living cell percentage was acquired $p = 0.001$ smaller from $\alpha = 0.05$ which means there was a quite significant difference between acrylic resin being soaked with or without NaOCl, either processed by conventional method or microwave.

DISCUSSION

Sodium hypochlorite (NaOCl) is a high level disinfectant material which is broad spectrum, effective to bacteria, spora, yeast, HIV and Hepatitis. The substance which has concentration of 0.5% can be used as disinfectant of acrylic resin denture.⁹ Because the majority of removable denture material is acrylic resin which can absorb fluid, it is feared that NaOCl will be absorbed by acrylic resin. To make a prove, toxicity test in vitro was performed by using cell culture method.

In this research the number of cell was calculated by direct counting on the number of living cell and death cell by hemocytometer. Living cells do not absorb stain of trypan blue, while dead cell absorbed the stain. After the calculation was done, the average number of living cells from all of the 5 groups was acquired. From all those four group, resulting the average mean of living cells between (95.43 ± 0.72) to (95.86 ± 0.11).

The percentage of living cells of all the group of acrylic resin which was soaked by NaOCl compared to the control group showed significant difference, except for group number 4 which was processed by microwave method. It happened because microwave polymerization produced less residual monomer compared to conventional method.¹⁰

On conventional polymerization, the heat energy derived from outside, and it caused monomer molecules outside which the accepted heat and continued the heat to the monomer molecules inside. The monomer molecules moved passively because of the heat from outside, therefore the process of polymerization started from outside to inside. This process caused the residual monomer trapped inside the mixture, which caused the residual monomer on the acrylic resin.¹¹ On microwave polymerization, the heat resulted as the effect of a very fast movement of the monomer molecules from a high frequency electromagnetic, where the crashes of inter molecular occurs and creating heat from inside to outside. The direction of the energy reduced the possibility of the residual monomer being trapped inside acrylic resin. Residual monomer is the monomer which is not reacted with the polymer, which eventually have the potentio to irritate the mucous tissue of the mouth,^{12,14} which determine that acrylic resin with microwave polymerization is more compatible compared to the conventional method.

Acrylic resin which was processed either by microwave or conventional method which was then soaked in NaOCl 0.5 % show significant difference. It was shown that by the soaking process, the percentage of living cells was reduced, which explain that NaOCl is a toxic disinfectant for the tissue. NaOCl is toxic and could destroy the cellular tissue. The content of chlorine in NaOCl solution acted fast and very effective to HBV and HIV. Chlorine can cause irritation to the skin or mucosa, because chlorine is able to release the free oxygen which will enter the protoplasmic cells which will destroy cells. The combination of chlorine with the membrane cells will form N-chloro compound which will disturb the metabolism of the cells. The changing of membrane cells, will cause diffusion that make the cell content come out, beside it can also destroy the membrane cell mechanically.⁵ The death of cells also caused by chlorine oxidation process in SH group and important enzyme, and it can cause the dysfunction of enzymatic process.³

This research used 0.5% NaOCl concentration. Neidle¹⁵ argued that the concentration can be used as antiseptic for membrane mucosa. Contradiction with Neidle, Mehra⁵ determined that the limit of toxic for tissue is 0.25%, but disinfectant of NaOCl with 0.5% concentration is very effective against Hepatitis virus and HIV beside yeast, parasite and spores. According to Mehra⁵ the concentration (0.5%) has reached toxic limit, and there is a possibility of residual solution of NaOCl which can cause tissue toxicity, so the percentage of living cells on tissue culture would be reduced.

The percentage of acrylic resin living cells which was processed by microwave method and soaked in NaOCl is higher than acrylic resin which was soaked in NaOCl and processed by conventional method. Even though the effect of soaking process decreased the percentage of living cells, acrylic resin which was processed by microwave resulting higher percentage of living cells compared to acrylic resin which was processed by conventional method, because at the beginning the residual monomer of acrylic resin is lower. It showed that the decrease of the number of living cells was because of the effect of NaOCl solution with chlorine.

The result of all toxicity test showed that the average mean of living cells which was processed by two methods of polymerization and being soaked in NaOCl solution resulting the mean more than (95.43 ± 0.72) which was closer to 100%, so it could be said that resin which was processed by both ways either being soaked or not resulting good biocompatibility. Material which has good biocompatibility has to be closer or equal to 100% the average mean of living cells percentage, or 92.3–100%.^{14,17}

The result of this research was acrylic resin denture which either being soaked or not by NaOCl fulfilled the condition. It is important to remember that the procedure of NaOCl 0.5% usage as disinfectant is by soaking the denture for 10 minutes and then rinsed it off. So the NaOCl will not make a contact with mucosa tissue directly, then NaOCl might cause less toxicity reaction to the oral mucosa.

It concluded that acrylic resin which was processed with microwave and soaked in NaOCl was more biocompatible compared to the conventional method which was soaked in NaOCl. It means that NaOCl 0.5% is biocompatible as removable denture disinfectant by means of soaking for 10 minutes and then rinsed. The usage of NaOCl 0.5% as cleanser for denture acrylic resin which was processed by microwave either by conventional method has to be done in a careful instruction, guaranteed that it is rinsed as clean as possible. It was found out in the research of culture cell media that the decrease of the number of living cells still occurred, although it was compatible.

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