Biocompatibility of acrylic resin after being soaked in sodium hypochlorite

Nike Hendrijatini
Department of Prosthodontic
Faculty of Dentistry, Airlangga University
Surabaya - Indonesia

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 polymeration and soaked in sodium hypochlorite, the second group was acrylic resin plate with microwave polymeration 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 recorded. 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

INTRODUCTION

Biocompatibility is a harmonius condition without any toxic effect on biological function, which is measured according to local cytotoxicity, systemical response, and carcinogen characteristic. The treatment of a denture is by taking the denture off and soaking it at night, beside the reservation and cleaning 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.

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. 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, 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. 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), 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 has been set, apply all the surface of gypsum with
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.

Acrylic resin plates were rinsed 3 times with aquadest,
and sterilized with UV Lights for 1 hour inside laminar
flow. Then the plate was stucked 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
tryptsin 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
hemosimeter, the number of living cells and dead cells
was calculated under the microscope, using the Bird and
Forrester method (Figure 2).
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.

<table>
<thead>
<tr>
<th>Number of sample</th>
<th>Living Cells Percentage</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Means</td>
</tr>
<tr>
<td>K</td>
<td>7</td>
</tr>
<tr>
<td>K–N</td>
<td>7</td>
</tr>
<tr>
<td>M</td>
<td>7</td>
</tr>
<tr>
<td>M–N</td>
<td>7</td>
</tr>
<tr>
<td>control group</td>
<td>7</td>
</tr>
</tbody>
</table>

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 (96.48 ± 0.52) was 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

<table>
<thead>
<tr>
<th></th>
<th>K</th>
<th>K–N</th>
<th>M</th>
<th>M–N</th>
</tr>
</thead>
<tbody>
<tr>
<td>control group</td>
<td>0.022 *</td>
<td>0.002 *</td>
<td>0.009 *</td>
<td>0.001 *</td>
</tr>
<tr>
<td>K–N</td>
<td>-</td>
<td>0.009 *</td>
<td>0.001 *</td>
<td>0.064</td>
</tr>
<tr>
<td>M</td>
<td>-</td>
<td>-</td>
<td>0.001 *</td>
<td>0.001 *</td>
</tr>
<tr>
<td>M–N</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

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 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 shows that there is a 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. 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 hemositometer. 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 (97.86 ± 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.³

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 potentiation 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 protoplastic 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 sporas. 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 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.

REFERENCES