Immersion of Alginate Impression (Hydrocolloid Irreversible) On Two Percent Glutaraldehyde Prevent Contamination of Mycobacterium Tuberculosis on The Stone Cast

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Objectives: to observe the effect of alginate impression (hydrocolloid irreversible) immersed on 2% glutaraldehyde on preventing contamination of *Mycobacterium tuberculosis* contamination on stone cast.
Methods: This is an experimental research with post test only control group design, with alginate impression material (hydrocolloid irreversible) is immersed in the 2% glutaraldehyde for 10, 15, and 25 minutes.
Results: This result shows that there is no *Mycobacterium tuberculosis* contamination on the stone cast after the immersion of alginate impression material for 10, 15, and 25 minutes. Immersion of alginate impression material (hydrocolloid reversible) in the glutaraldehyde 2% for 10 minutes is effective to prevent *Mycobacterium tuberculosis* contamination on the stone cast.
Conclusions: Immersion of alginate impression on 2% glutaraldehyde for 10 minute was effective to prevent contamination of *Mycobacterium tuberculosis*
Keywords: Tuberculosis, *Mycobacterium tuberculosis,* alginate impression material, glutaraldehyde

INTRODUCTION

Tuberculosis is one of infectious disease cause of death and until tody this disease still remains a problem worlwide, including Indonesia. Therefore, the disease is known as The Re-emerging disease.

World Health Organization (WHO) stated that there was 2 million people infected by *Mycobacterium tuberculosis*. Indonesia is the third ranking of Tuberculosis after China and India in the world. These three countries contribute 50 % Tuberculosis patients in the world. A new Tuberculosis case in Indonesia is 583,000 people per year.

A denstist is in high risk to infect or to deliberate *Tuberculosis* among patient, dentist, nurse, assistance, and dentist laboratory staff. Deliberation is generally through saliva and blood in either direct or indirect contact. Effort to protect deliberation can be through wearing mask, glove, and sterilization of the instrument used. Deliberation could also be through casting material applied on dentist practice.[1]

During dentist practice casting procedure or model are two important thing. Varies casting and model can be formed from gypsum product by using mold and or negative reproductive to support the gypsum. Preparing stone cast is a part of dentist work, including construction of labil protesa or cast. One of casting material is alginate. Alginate is an hydrocolloid irreversible material which is applied through solve to water and its transformation could not return to its initial form (irreversible).

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Treated casting material will be casted to the patients mouth and contaminated with saliva patients. Application of this alginate is much more applicable compare to other casting materials. Some researchs report that *Stapilococcus aureus, Esceria coli* and *Candida albicans* are survive on alginate casting.[2] Oral microorganisms grow and develop on alginate casting.[3] Other researchs indicate that these microorganisms have an ability to travel from the alginate to the stone cast.[4] Alginate was contaminated by *Escherichia coli, Stapilococcus aureus, Enterobabacter claocea, Pseudomonasnseruginosa, Klebsiella pneumonise, Actinobacter calcoaceticus, Bacillus sublilia, Mycobacterium phlei*, and *Candida albicans*.[5] Ray and Fuller (1963) found that 12% of alginate casting material contaminated by *Mycobacterium* tuberculosis on patients with tuberculosis.[6] Stone cast could also contaminated with *Streptococcus sanguis, Streptococcus salivarius*, and *Pseudomonas aeruginosa*.[7] In simple word, alginate casting material can be said as a cross infection media. When this casting material was processed to further process in laboratory its also potent to deliberate bacteria or virus to the surface model formed, therefore, model formed with gypsum will also potent as a cross infection media.[1,4] Stone cast made of alginate and contaminated with infectious microorganism deliberate to laboratory staff during cast trimming or through inhalation during breath.[8]

This research aims to observe application through immersion of alginate to 2% of glutaraldehyde for protecting *Mycobacterium*

tuberculosis contamination on stone cast. In this study, immersion time was also determine.

METHODS

This study is applying an experimental with post test only control group design. Research was carried out at Microbiology Laboratory Sanglah General Hospital for 3 months.

Rejuvenation of *Mycobacterium tuberculosis*

Mycobacterium tuberculosis H37RV supplied by Clinical Microbiology Installation of Sanglah General Hospital were cultured on Lowenstein Jensen media. Cultured *Mycobacteruim tuberculosis* on Lowenstain Jensen media were then suspended on aquadest (0.0076 g on 7.6 mL). This is equivalent to turbifity of 1 Mac-Farland scale.

Procedure

Alginate made of a mixture of plaster of Paris and water (6.5 g : 1.5 mL) was mixed thoroughly and then casted on the spoon mold. Then, the mold was separated and the casting was immersed on Mycobacterium tuberculosis suspension. All treatment was divided into 4 groups, i.e. the first group is a control group without immersion, the second group is group immerse with 2% glutaraldehyde for 10 minutes. Then, the third group is immersed on 2% glutaraldehyde for 15 minutes. The last group, the fourth group is immersed on 2% glutaraldehyde for 25 minutes.

Statistical Analysis

All data were descriptively analyzed and followed by ANOVA test for obtaining the treatment different. Values of $p<0.05$ were considered to be indicative of statistically significant different.

RESULTS

In this study, evaluation of *Mycobacterium tuberculosis* contamination on *stone cast* was carried out after 6 weeks of culture. The research shos that there was a growth of *Mycobacterium tuberculosis* colonys only on control group. Meanwhile, for all treatment groups there were no growth of *Mycobacterium tuberculosis* observed. Data of *Mycobacterium tuberculosis* growth were listed on Table 1.

Tabel 1 reveals that there were no growth of colony observed for treatment groups. That means immersion on 2% glutaraldehyde for 5, 10, and 25 second have already kill all bacteria. In simple word, immersion of the mold on 2% glutaraldehyde for 5 minute have already killed the bacteria. Therefore, based on data on Table 1, normality and homogenity test were neglect. Afterwards, ANOVA was applied to determine the different of treatment. Anova test indicates there was a significant different among control and treatment group (p=0.001).

Table 1
Growth of *Mycobacterium tuberculosis*

No	Groups (colony)			
	I	II	III	IV
1	109	0	0	0
2	105	0	0	0
3	115	0	0	0
4	117	0	0	0
5	112	0	0	0
6	104	0	0	0
Average	110.33± 5.28	0	0	0

Remarks: Group I = control, Group II = treated with 2% glutaraldehyde and immersed for 10 minutes, similar treatment was applied to others two groups III and IV, instead of immersion time, 15 and 25 minute, respectively.

DISCUSSION

Lowenstain Jensen Media

Lowenstain Jensen (LJ) media is a media for isolation and identification of *Mycobacterium.* This media is reach of nutrient and selective, differentiate, for *Mycobacterium tuberculosis.* In this media, green malachite was used to inhibit other bacteria and modified using citrate and phosphate. Glyserol sources from carbon and energy is needed for *tubercle bacillus* human type. Asparagin and RNA were grown to provide nitrogen source and growth stimulant taken from albumin during the process. In this study, this media is chosen because such media is a conventional standar method for detecting *Mycobacterium tuberculosis*.[9]

Rejuvenation of *Mycobacterium tuberculosis*

Rejuvenation was carried out by making suspension of *Mycobacterium tuberculosis* H37RV on water and culture to LJ media incubated for three weeks at 37ºC. After incubation, dry rough yellow brownish colonies were obtained. Furthermore, the colonies obtained were characterised with irregular form, protruding, and their surface are wrinkles such as cauliflower, rod like form, 5µm long and 3µm wide. Therefore, the colonies obtained are positive *Mycobacterium tuberculosis* H37RV. These bacteria do not form a spore, aerob, and a gram positive bacteria, and acid-resistant bacteria.[10,11]

Mycobacterium tuberculosis are bacterial pathogens cause of tuberculosis, a dangerous and even deadly disease. The bacteria deliberate to the dentist through inhalation of aerosol/droplet from oropharyngeal secretion and saliva.[1,,12,13] These

bacteria were growth in groups, therefore, they were more chemical resistant compare to other bacteria. Tubercle bacillus can live at temperature between 30ºC – 40ºC, resistant to dryness and can live for long periods (8 – 10) days in dry sputum attach to dust.[10,14] The bacteria, due to their renic size could be inhaled to alveolus and achieve lobus alveolus below the lung.[15]

Alginate Casting Material

Alginate casting material is a hydrocolloid irreversible casting material to be used for making jaw duplication model. The use of alginate casting material far beyond the other casting materials. Manipulated casting material will be casted into the patient's mouth and contaminated with the patient's saliva.[16,17] Processing this casting material at laboratory is potential to transfer bacteria or virus to the formed surface model which was made of gypsum. Therefore, this model is potential as a cross infection media to deliberate tuberculosis.[1,4,8] Contaminated alginate casting material with infectious microorganism could deliberate to staff of laboratory during the *triming* or dies processing.[8]

Contamination of *Mycobacterium tuberculosis*

Contamination of *Mycobacterium tuberculosis* observation for all groups was employed after the first 3 weeks cultured. The results indicates that the bacteria growth was only observed on control group. On the other hand, for the other three groups II, III, and IV, there were no growth of bacteria observed. To assure that there were no growth on treatment groups, the growth observation was continually observed for 6 weeks. During 6 weeks culture, it was also observed that there were no growth for all treatment groups. In addition, for control group the growth become much more wider compare to the other groups.

In this study, it was obtained that there was a groth of bacteria on LJ media of control group without immersion on 2% glutaraldehyde. Therefore, there was a contamination of *Mycobacterium tuberculosis* on stone cast in this group. However, no bacteria growth were observed on the treatment groups which were immersed on 2% glutaraldehyde for 10, 15, and 25 minutes. This indicates there were no contamination of *Mycobacterium tuberculosis* on the stone cast.

Shofou *et al*, (2002) reported that from 107 casting material alginate (hydrocolloid irreversible) samples at dental laboratory at Sweden, only 50% of them using disinfectant to wash their casting material and 50% of them only use tap water to wash their casting material.[18] Shofou and colegous use questionnaire to gain data from their samples. They also reported, that there was no different of bacteria growth between washing with disinfectant and tap water. In their research was not mentioned of the kind and how long of disinfectant used.

The choice of 2% glutaraldehyde to prevent contamination of *Mycobacterium tuberculosis* on stone cast are for some reasons, including this material function as denaturation in combination with 0,5% - 5%. Furthermore, glutaraldehyde has more effective reaction compare to formaldehyde, therefore, much more chosen as alginate material compare to other material in virology and does not have a cariogenic effect. Treshold value of glutaraldehyde is 0.1 mg/L. Mechanism of how glutaraldehyde destroy microorganism is through protein or nucleic acids alkylation .[19] Principally, glutaraldehyde can be used in wide spectra application, besides its ability to kill microorganism, it can also destroying virus, sporacide, and does not corrode metals. In contrast to formaldehyde, which is can only destroy microorgasnism and also corrosive. In addition, glutaraldehyde are also stable, persistence, and can destroy vegetative bacteria in two minutes.[19,20]

Solution of 2% Glutaraldehyde pH 7.5-8.5 is a high level disinfectant (HDL) and can be used for desincfect a certain medcal instrument that could not be sterilized.[19] When compared with other HDL desinfectants, such as hydrogen peroxide (H_2O_2) is not stable in an open container changes to water. In addition, phenolic desinfectasns are corrosive to rubber and plastic used in medical treatment and did not acts as a sporocide.

Centers for Disease Control and Prevention, (2008) conducted a research regardless of the use 2% glutaraldehyde on endoscopic tool for Hepatitis-B Virus (HBV), Hepatitis-C Virus (HCV), Human Immunodeficiency Virus (HIV), and *Mycobacterium tuberculosis*. The results indicate that there was no contamination on those instrument used.

In our study, it was obtained that there was a significant different between alginate casting material without immersion on 2% glutaraldehyde (control) and treatment with immersion on 2% glutaraldehyde ($p < 0.05$). However, no significant different observed among treatment in accordance to the length of immersion, i.e. 10, 15, and 25 minutes. Amazingly, no contamination of *Mycobacterium tuberculosis* observed in all groups of treatment. In simple words, immersion of alginate casting materials for 5 minutes have already destruct the bacteria.

As mentioned above, mechanism of glutaraldehyde to destroy bacteria are as protein or nucleic acids denaturation. Protein denaturation is a protein structure changes or modifies, in term of secondary,

tertiary, and quatenary without breaking covalent bonds. In simple words, denaturation can be defined as breaking of hydrogen bond, salt bridge, and opening the protein molecul crease. Denaturation is a reversible process, that can be return to its initial form when the cause was reduced.[21]

Rohani (2010) stated that glutaraldehyde immerion time needed to inactivated of *Mycobacteriu.*sp was around 20 – 30 minutes.[19] Other researchers obtained the immersion time required was around 10 – 20 minutes.[22] Generally, casting materials were not changes after immersion between 30-60 minutes.[2] Therefore, based on this point of view in this study immersion time applied were in the range of 10, 15, and 25 minutes.

In this study, it was obtained that there was no different among immersion time period of 10, 15, and 25 minutes. This is due to immersion for 10 minutes have already resulted in inhibition of reversible protein denaturation.

In addition, solution of 2% glutaraldehyde has an ability to destroy vegetative bacteria, including *Mycobacterium tuberculosis*. These type of bacteria were characterized by thick cell wall which is compromised of *lipoarabinomanan*. This cell wall plays an important role on interaction of host and phatogens, so that it could resist on macrophage attack.[10,23]

CONCLUSION

Immersion of alginate casting materials with solution of 2% glutaraldehyde for 10, 15, and 25 minutes inhibit contamination of *Mycobacterium tuberculosis* stone cast.

Immersion of alginate casting materials with solution of 2% glutaraldehyde for a periods of time, 10, 15, and 25 minutes did not result in a different contamination of *Mycobacterium tuberculosis* effect on stone cast.

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