# The Effect of Chitosan and Acrylate Acid Complex into Acrylic Resin as Denture Material Against Fibroblast and Inflammatory Cells

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**Abstract**

This study investigates the effect of chitosan and acrylate acid addition in the acrylic resin as denture material toward the number of fibroblasts and inflammatory cells including macrophages, lymphocytes, and plasma cells.

This study is an experimental laboratory research. Nine Wistar rats (Rattus Novergicus) were divided into 3 groups (n= 3). Control group receiving treatment without mixture of acrylic resin (CO), group 2 acrylic resin mixture with chitosan and 1% acrylate acid (ARC1), while group 3 acrylic resin mixture with chitosan and acrylate acid 2% (ARC2). The acrylic resin plate is formed in 4 mm-sized discs with 2 mm thickness. Resin samples were implanted into subcutaneous dorsal-area of Wistar rats, then stitched. Microscopic evaluation conducted on day 7 post-implantation. Histological examination combined hematoxylin eosin staining and 400x magnification light microscope. Fibroblasts, macrophages, lymphocytes and plasma cells were counted using the Image J program. Data were analyzed using one-way ANOVA and followed by LSD test with 95% level of significance.

Results revealed that fibroblasts, macrophages, lymphocytes and plasma cells count in the CO group were higher than the ARC1 and ARC2 groups. Greater concentration of chitosan and acrylate acid in the acrylic resin decreased the mean of fibroblasts, macrophages, lymphocyte and plasma cells. One-way ANOVA test presented significant differences in the fibroblasts, macrophages, lymphocytes and plasma cells count of the control and treatment groups ($p<0.05$). The LSD test indicated significant difference between CO and ARC2 group.

We concluded that the combination of acrylic resin with chitosan and 2% acrylate acid concentration is properly perceived by rat's body tissue.

**Experimental article (J Int Dent Med Res 2021; 14(4): 1459-1464)**

**Keywords:** Acrylic resin, acrylate acid, chitosan, fibroblast cells, inflammatory cells.

**Received date:** 30 August 2021 **Accept date:** 24 September 2021

## Introduction

A healthy oral cavity without complete structure of teeth affects individual wellbeing. Partial or complete loss of tooth resulting imperfect mastication which disrupt the digestive system. The appropriate resolution of this problem is replacing the missing teeth with dental prosthesis (dentures).[1] Plate is the part of prosthetic device that always engaged with the oral tissue under long-term use so the dental materials must have comprehensive properties and good biocompatibility to function properly.[2] Base plate of the denture predominantly origin from acrylic resin material. Ideal denture material required to meet mechanical and biological properties.[3] However, the acrylic resin present residual monomers that cause sensitivity reactions in the oral mucosa, including allergic reactions, mouth soreness, burning sensation, irritation to the oral mucosa and cytotoxic effects.[4] Besides, acrylic resin is hygroscopic and porous which causing a rough surface and affects the mechanical strength. This surface promotes bacteria and fungi colonization, causing inflammation to the oral tissues.[5] It is necessary to select appropriate material that

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avoids microorganism growth and strengthens mechanical properties of the acrylic resin.

Natural materials have been extensively used, researched, and produced for medical purposes in recent years.[6] Chitosan derives from shrimp shell extract which is processed through demineralization, deproteination and deacetylation, and attributed to fungistatic, biodegradable, biocompatible, non-toxic properties.[7] Acrylic resin can be mixed with chitosan within the assistance of acrylic acid coupling agent. When mixed with chitosan, acrylic acid produces a complex interpenetrating network structure, resulting strong adhesion between surfaces.[8] The interaction between acrylic resin and chitosan form new bonds through free radicals on the residual monomer of acrylic resin.[9] Research studies has been identified that a mixture of acrylic resin with chitosan and acrylic acid at 1% and 2% concentrations is non-toxic.[10]

Various dental material supplies generate different responses to the surrounding cells and tissue. Therefore, the biocompatibility of a dental material needs to be verified. Biocompatibility evaluation carried out by implantation of foreign materials or research samples to the subcutaneous tissue of experimental animals. This treatment causes wound or injury to the body tissue of experimental animal. Any kind of physical injury initiate a series of enzymatic and cellular reactions to the damaged tissue. The reaction recognized as inflammatory response, which occurred in the early phase of tissue repair and regeneration process.[11] Principal process in the biological body compensation towards wounds and injuries consist of several phases, including inflammation, proliferation, and remodeling.[12]

Inflammation is a biological response from the immune system that can be triggered by various factors, including pathogens, damaged cells, and toxic compounds. Uncontrolled acute inflammation leads to chronic inflammation.[13] Cells that play a role in tissue inflammation are macrophages, lymphocytes and plasma cells. Macrophages operate as the main regulators in inflammation and fibrosis, also serve as phagocytes to eliminate apoptotic cells and activate lymphocytes for the immune response.[11] Lymphocytes are white blood cells uniform in appearance but varied in function and include T, B, These cells are responsible for innate and immunity antibody production.[14] Plasma cells are a type of inflammatory cell derived from B lymphocytes in a secondary immune response to provide rapid antibody production.[15] Fibroblasts commenced to respond and proliferate. The number of fibroblast cell proliferation correspond to the active inflammation of inflammatory cells in the tissue. The higher number of fibroblasts proliferation subsequently increased inflammatory cells count and resulting higher toxicity. The tissue response mechanism against foreign bodies can be determined from the number of fibroblasts and inflammatory cells.[16] This study aimed to examine the effect of acrylic resin with chitosan and acrylic acid concentrations of 1% and 2% matrix mixtures as a base material for dentures toward the number of fibroblasts, macrophages, lymphocytes and plasma cells.

## Materials and methods

This study is a laboratory experimental research. This research has been accepted by the Research Ethics Commission of the Faculty of Dentistry, UGM, with the registration number: 00134/KKEP/FKG-UGM/EC/2019. A total of nine male Wistar rats aged 8-15 weeks weighted 230-300 grams involved in this study. Rats were divided into 3 treatment groups: control group and 2 treatment groups. Each group consists of 3 rats. Acrylic resin disc-shaped plate, with 4 mm diameter and 2mm thickness sterilized for 15 minutes in 134 $^0$C temperature by autoclave. The disc soaked in Virkon™ solution for 20 minutes and rinsed with $H_2O_2$ 3%. Experimental animals were injected by intravenous anesthesia, combination of xylazine (95mg/kg) and ketamine 10% (25mg/kg). The dorsal rats' hair were shaved and smeared with povidone iodine. When the rats under anesthesia, ±5mm length incision was made to penetrate the subcutaneous tissue. Acrylic resin plates were implanted on the rear, left and right of the rat's back following sequence of Group 1 (control) on the back, Group 2 and 3 on the right and left respectively. 3 interrupted stitches of polyglycolic acid threads were sutured and iodine as an antiseptic was applied. Furthermore, the rats were enclosed in individual cages and observed for symptoms, behavioral changes and death likelihood for 24 hours. In addition, observation was intended to identify

whether edema, hematoma, erythema and signs of inflammation occurred. Following 7 days of treatment, all experimental animals were terminated in this study by injection of overdose general anesthesia.

To obtain histological preparations, the underneath tissue of implantation sample was taken and isolated by immersing in 4% para formaldehyde solution, stored in labeled container. Later, histopathological examinations were performed. Tissue samples stained with eosin hematoxylin and observed by optical microscope with 400x (40x objective lens, 10x ocular lens) magnification. Each sample observed from at least 3 fields of view. Fibroblasts, and inflammatory cells calculation including macrophages, lymphocytes and plasma cells performed on observed sample photos using Image J program. Photos of the samples imported to Image J program. Different color applied to distinct each cell and avoid duplication. The average count of fibroblast and macrophages, lymphocytes and plasma cells were calculated. A homogeneity test with Levene's test was carried out to determine differences among fibroblasts and macrophages, lymphocytes and plasma cells count between control and intervention groups. One-way ANOVA test was performed ($p<0.05$) followed by post hoc LSD.

## Results

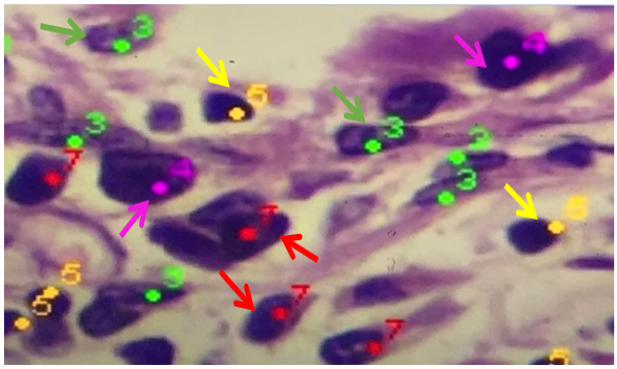

**Figure 1.** Subcutaneous histopathological imaging of the wistar rats.
: Fibroblast cells
: Macrophage Cells.
:Lymphocyte cells
:Plasma Cells

Observation 24 hours after implantation of the sample shows a mild inflammation in the form of erythema. Cell counts were carried out with the help of the imageJ program on light microscope examination with 400x magnification in 3 different fields of view. Figure 1 shows an overview of the results. The histopathological imaging of matrix mixture combination of acrylic resin with chitosan and 1%-2% acrylate acid presented in Figure 2-4.

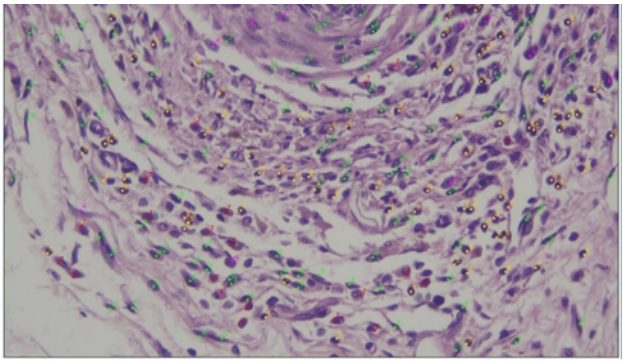

**Figure 2.** Subcutaneous histological of Wistar rats after application of acrylic resin plate (control).

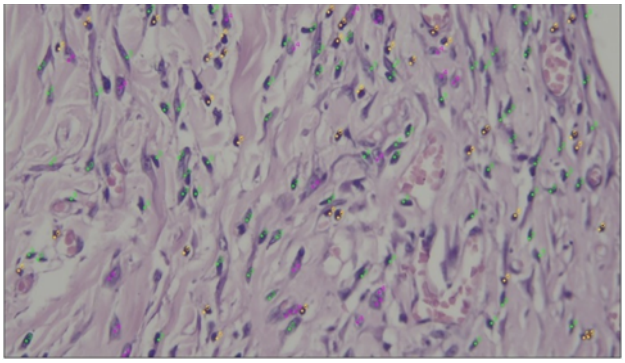

**Figure 3.** Subcutaneous histopathological of the Wistar Rats after application of matrix mixture combination of acrylic resin with chitosan and 1% acrylate acid.

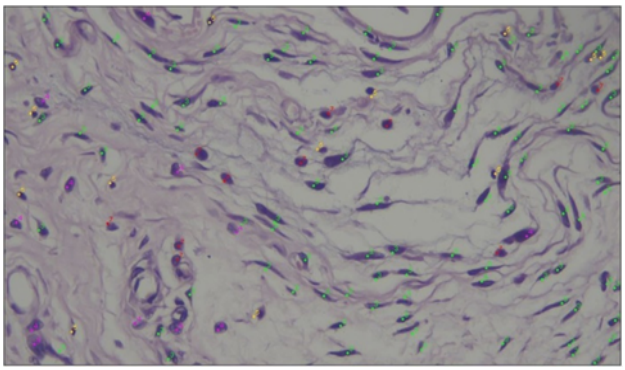

**Figure 4.** Subcutaneous histopathological of the Wistar rats after after application of matrix mixture combination of acrylic resin with chitosan and 2% acrylate acid.

Figure 2-4 show a decrease in the number of inflammatory cells (macrophage cells, lymphocyte cells, plasma cells and fibroblast cells, respectively from control group, then mixture acrylic resin, chitosan with 1% acrylic acid, and mixture acrylic resin with chitosan and 2% acrylic acid.

Mean and standard deviation calculation of inflammatory and fibroblasts cells of each group presented in Table 1.

| No | Group | Fibroblast | Macrophage | Lymphocyte | Plasma Cells |
|---|---|---|---|---|---|
| 1 | Control | 83.06 ± 17.09 | 30.66 ± 8.09 | 28.55 ± 10.45 | 5.77 ± 2.23 |
| 2 | ARC 1% | 75.14 ± 11.31 | 21.27 ± 6.90 | 15.81 ± 4.33 | 4.18 ± 1.78 |
| 3 | ARC 2% | 64.26 ± 9.72 | 18.46 ± 3.53 | 13.24 ± 7.49 | 3.00 ± 1.95 |

**Table 1.** Mean and standard deviation ( $\bar{x}$±SD) of fibroblasts, macrophage, lymphocyte and plasma cells count (HPF)
Notes: ARC = mixture of acrylic resin, chitosan and acrylate acid.

Table 1 indicated that the highest average of inflammatory cells (macrophages, lymphocytes, plasma cells) and fibroblasts cells acquired from acrylic resin group (control group). The average cells count decreased after the addition of chitosan combined with 1% and 2% acrylate acid. Highest inflammatory cells reduction identified at 2% concentration presented by fibroblasts 64.26 ± 9.72, macrophages 18.46 ± 3.53, lymphocytes 13.24 ± 7.49 and plasma cells 3.00 ± 1.95

Before one-way ANOVA (α=5%), Shapiro-Wilk homogeneity test was carried out and indicated normal data distribution by p>0.05. Levene's homogeneity test presented fibroblasts 0.35, macrophages 0,06, lymphocyte 0,25, and plasma cells 0,49. p value reported > 0.05, indicate homogeneous data. One-way ANOVA among inflammatory cells count of and fibroblasts, macrophages, lymphocytes and plasma cells between three groups presented in Table 2.

| Variable | F count | F table | *p value* |
|---|---|---|---|
| Fibroblasts | 4.67 | 3.04 | 0.011* |
| Macrophages | 8.76 | 3.04 | ≤0.001* |
| Lymphocytes | 9.85 | 3.04 | ≤0.001* |
| Plasma cells | 4.36 | 3.04 | 0.021* |

**Table 2.** One-way ANOVA Test Results.
*) =F count ≥ F table or p ≤ 0.05 indicate significant results.

One-way ANOVA test provide value of F count higher dan F table and p value below 0.05 among inflammatory cells (macrophages, lymphocytes, plasma cells) and fibroblasts. This calculation confirmed that significant effect identified from the intervention as evidenced by 1% and 2% acrylate acid combined with chitosan from the acrylic resin denture base affects fibroblast, macrophage, lymphocytes, and plasma cells count. Post-Hoc LSD test indicated no significant differences between control and 1% intervention group, while control and 2% mixture concentration indicated significant difference (p≥0.05).

## Discussion

The inflammatory reaction is an initial response to the presence of a cell or tissue injury caused by stimulus. The inflammatory reaction is a defense mechanism for the body against foreign objects that infiltrate the human body.[17] In accordance with this theory, the study implanted stimulus in the form of acrylic resins added with chitosan and acrylate acid on the dorsal area of Wistar rats. Observations took place on the 7th day of research procedure when the neutrophil cells were not identifiable since the initial reaction due to death occurred on the third day correspond to the elimination of microorganisms that enter the body. Observations were not carried out on day 14 because the sample could be wrapped in a collagen capsule.[18] Control and intervention groups in this have been detected fibroblasts and macrophage, lymphocytes and plasma cells. The responses were initiated by injuries due to tissue damage which will develop into homeostasis phase and inflammation, proliferation and maturation. It is reported that inflammation process can be triggered by various factors including pathogens, tissue damage and toxic compounds.[13] The occurrence of inflammation may also be caused by residual monomer compound from acrylic resin which not completely involved in the polymerization process. Polymerization process of acrylic resins produce residual monomer which stimulates fibroblast cell proliferation that induce inflammatory response resulting tissue irritation, allergic reactions and cytotoxic.[19]

Inflammatory cells among control group was greater than the intervention group, as presented in the mean cells count of fibroblasts, macrophages, lymphocytes and plasma cells. The fibroblasts, and macrophage, lymphocytes and plasma cells count decreased in the

treatment group 2 acrylic resin mixed with chitosan and 1% acrylate acid, and group 3 at a concentration of 2%. This phenomenon is stimulated by the addition of chitosan with biocompatibility properties[8] and the chance of decreased residual monomers because free radicals in the acrylic resin during the polymerization process bond to form a complex interpenetrating network structure[9]. Decreased fibroblasts and inflammatory cells count indicates healing process consistent with the pathogenesis of wound healing. At the final stage of healing process, fibroblasts and inflammatory cells apoptosis, capillary growth stops, blood flow to the wound area and metabolic activity decreases.[20]

In this study, the fibroblasts cell count was greater than the other inflammatory cells. This is due to the presence of components from a matrix of mixtures of acrylic resin with chitosan and acrylate acid which affect the inflammatory process, so that fibroblast cells proliferate. When an injury occurs, fibroblasts appear to be more active in producing extracellular matrix and indicate end point of tissue repair.[21] At the time when the sample remain in the body of the experimental animal and cannot be destroyed and neutralized, in a few minutes to a few days an acute inflammation occurs. This inflammation phase is characterized by vascular changes, fluid and plasma proteins exudate. In the later phases acute inflammation can develop into a chronic inflammation if the response does not subside, and if the injury agent or injury remains in the tissue. Chronic inflammation in this study can be identified by the presence of inflammatory cells, including macrophage cells which have the highest cell count, followed by lymphocytes, plasma cells and blood vessel proliferation, and within 7 days period. Chronic inflammation lasts longer than acute response and associated with the severity of tissue damage caused by persistent stimuli, often lasts in weeks to months, depending on the size of the wound.[22]

The average count of macrophages in this study rank second after fibroblasts. Macrophages interact with lymphocytes to secrete inflammatory mediators such as histamine, serotonin, bradykinin, prostaglandins and leukotriene. Macrophages are monocytes compound in the blood which produce fibroblast growth. Lymphocytes stands in the third average count after macrophage cells. The response stimulated at the end of inflammatory process, mainly identified in chronic inflammation or at the end of inflammatory phase. There are 2 types of lymphocytes, B cells and T cells. T cells promote the formation of antibodies, inhibit immunoglobulin synthesis and correspond as cytotoxic cells. Plasma cells were lowest average count in this study. At the response of an injury event, vascularization process associated with capillary permeability where blood and fluid substances leave the plasma to approach the injured area. Plasma cells originated from B lymphocytes that produce antibodies to encounter antigen. Lower count of plasma cells is associated with fewer number of B lymphocyte compared to T lymphocytes and other mononuclear cells.[23] B lymphocytes identified with the lowest number during inflammatory process.[24]

One-way ANOVA confirmed a significant difference due to the addition of chitosan and acrylate acid in the acrylic resin as denture base to the number of inflammatory cells (macrophages, lymphocytes, plasma cells) and fibroblasts. Combination of chitosan and acrylate acid 1% and 2% concentration enhance the materials assembly, improve the polymerization process and reduce residual monomer. Residual monomer stimulate an inflammatory response.[25] Material biocompatibility determined by residual monomer that released through dissolving process. Residual release cause cell damage and stimulate the synthesis of certain proteins such as inflammatory mediators (interleukin-1 and 6), but also influenced by superficial material absorption, protein accumulation, cellular matrix material interactions and biological adaptation to the material.[26] Post-hoc LSD test indicated significant difference between control group and the intervention groups. At the intervention group, combination of acrylic resin with chitosan and acrylate acid enhance resins structure. The denture has excellent porosity, smooth resin surface contact with the tissue provide better biological responses. Better tissue response toward foreign material, resulting lower count of inflammatory cells (macrophages, lymphocytes, plasma cells) and fibroblasts in the surrounding area. Smooth surface of a denture material provides better response to indirect injury to tissue.[27] Surface roughness of acrylic resin affect cell orientation and migration.[25] Furthermore, the acrylic resin matrix mixture with combination of

chitosan and 2% acrylate acid least stimulate inflammatory cells compared to other groups. This study results presented better response to foreign compounds.

## Conclusions

Combination of acrylic resin with chitosan and 2% acrylate acid concentration is properly perceived by body tissue.

## Acknowledgments

This work is supported by Damas Scheme, Faculty of Dentistry, Universitas Gadjah Mada.

## Declaration of Interest

The authors report no conflict of interest.

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