

Tissue reaction against implantation of nanocomposite and giomers

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ABSTRACT

Dentistry today has inherited technological advancement from other Dental Material sciences, examples are Nanocomposite and Giomers. Nanocomposites and Giomers are common materials used in Dentistry. But what are the implications when these materials are used in practice? The aim for this study shows the analysis of tissue reaction due to implantation of Nanocomposite and Giomers. The subcutaneous tissue of a mouse is substituted with the human gum tissues. In experimental group, Nanocomposite and Giomers were implanted in the subcutaneous tissue abdomen region in mice. The slides were made from the surrounding of implantation for both experimental and control groups. The evaluation of the effects of the implant is done in a time interval. Evaluated time intervals are 24 hours, 7th day, 14th day, 21st day and 28th day respectively. The amounts of inflammatory cells formation in both groups were compared. Once the results of the inflammatory cells are evaluated in the given time interval for Nanocomposite, Giomer and control group then they are statistically analyzed. The statistics used in the experiment are Mann-Whitney and Wilcoxon. The conclusion of this research showed that statistically significant differences on lymphocytes value between treatment and control group.

Key words: Nanocomposite, giomer, acute inflammation, chronic inflammation

ABSTRAK

Bidang kedokteran gigi pada masa kini telah mengalami berbagai perubahan dalam dunia teknologi dan pengetahuan, sebagai contoh Nanocomposite dan Giomers. Nanocomposite dan Giomers merupakan material yang biasa digunakan dalam bidang kedokteran gigi. Persoalannya adalah apakah implikasi material apabila digunakan dalam praktik? Studi ini menganalisa reaksi jaringan terhadap implantasi Nanocomposite dan Giomers. Jaringan subkutan mencit sebagai substitusi jaringan di rongga mulut manusia. Group percobaan, Nanocomposite dan Giomers ditanam dalam jaringan subkutan di bagian peritoneal (abdomen) mencit. Kemudian preparat dibuat pada tempat tertanam Nanocomposite dan Giomers dari kelompok perlakuan dan dari lokasi yang sama pada kontrol. Evaluasi reaksi terhadap implantasi dilakukan dalam waktu yang berbeda. Perbedaan waktunya adalah 24 jam, hari ke-7, 14, 21 dan 28. Jumlah sel-sel radang pada kelompok perlakuan dibandingkan dengan kelompok kontrol. Setelah mengetahui jumlah sel-sel radang dalam waktu yang berbeda untuk Nanocomposite, Giomers dan juga kelompok kontrol, kemudian dilakukan analisa statistik. Analisa statistik yang digunakan dalam studi ini

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adalah Mann-Whitney dan Wilcoxon. Kesimpulan penelitian ini berdasarkan hasil dari analisa statistik, limfosit menunjukkan perbedaan signifikan dalam kelompok perlakuan dan kelompok kontrol.

Kata kunci: Nanocomposite, Giomer, peradangan akut, peradangan kronis

INTRODUCTION

Any foreign body that enters into our body will cause and leads to tissue inflammation. Tissue inflammation is by definition of Inflammation (Latin, inflammation to set fire) is the complex biological response of vascular tissues to harmful stimuli, such as pathogens damaged cells or irritants. Inflammation can be classified as acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues.^{1,2}

Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells which are present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process. Causes of tissue inflammation includes burns, chemical irritants, frostbite, toxins, infection of pathogens, necrosis, physical injury, blunt or penetrating, immune reactions due to hypersensitivity, ionizing radiation, foreign bodies including splinters and dirt.^{1,2}

Inflammation is also intimately interwoven with repair processes whereby damaged tissue is replaced by the regeneration of parenchym cells, and or by filling of the residual defect with fibrous scar tissue. Although inflammation helps clear infections and along with repair, makes wound healing possible, both inflammation and repair have considerable potential to cause harm. Inflammation is divided into two basic patterns; they are acute and chronic inflammation.³⁻⁵

Acute inflammation is of relatively short duration, lasting from a few minutes up to a few days and is characterized by fluid and plasma protein exudation and a predominantly neutrophilic leukocyte accumulation. Chronic inflammation is of longer duration (days to years) and is typified by influx of lymphocytes and macrophages with associated vascular proliferation and scarring.³⁻⁵

Implicit in the definition of biocompatibility is an interaction between the body and the

material. Classically, these reactions have been separated into toxic, inflammatory, allergic and mutagenic reaction. Inflammation is second fundamental type of biological response to a material. The inflammatory response is complex but involves the activation of the host's immune to ward off some threat. Inflammatory may result from toxicity or from allergy and often the inflammatory response precedes toxicity.

The biocompatibility of glass ionomer cements is very important because they need to be in direct contact if any chemical adhesion is to occur. In a vitro study, freshly mixed conventional glass ionomer cement was found to be cytotoxic, but the set cement had no effect on cell cultures. The reason for furthering this research is based on the fact that in Operative Dentistry Class II and Class V which is widely use and the specified class is prepared more towards the soft tissue contact. In this research would be focusing on how the material would affect the soft tissue.⁶⁻⁸

In this modern age of technology which is fast growing there are many advanced materials which are used as filling materials, for an example, the Nanocomposite and Giomers. In this experiment would be focusing on the nanocomposite and Giomers against the tissue. There arent any further information given on books and resarch by researchers on these materials cause it is still a new product on use and it would be a great benefit by doing the further research on these study to know more about the material and its effect against the tissue.

In this research the key aim of experimental is on the biocompatibility (the reaction of the tissue), and focus of this research is more towards inflammatory. The research would be carried on Swiss Webster mice. Mice are to be known as a world wide usage for clinical experiment and the subcutaneous tissue can be use to observe changes in inflammatory cells. Moreover this research is being done in the subcutaneous tissue of the mice's abdomen because histological and embryological it has a similarity with a human gums.

METHODS

The material use in this experiment was experimental animal (in vivo), chemical materials and reaction material. Instrument use in this experiment was object glass, surgical instrument, microscope, and digital camera. Reaction material which is used is restoration material on a tooth which contains Nanocomposite or Giomers. The material was light cured for about 10 seconds on a fiberglass material with a length of 2 mm, width of 1 mm and the depth was 0.5 mm. The shape of the material was oval and kept in a air tight place.

The procedures of research were as follows: Equipments and material prepared for a research; Minor surgical tools, spatula, Giomers and Nanocomposite materials, glass lab, object glass and cover glass were sterilized in advanced. Thirty mice were divided into 2 groups; Group 1 served as the Nanocomposite group consisting of 15 mice and Group 2 consisting of 15 mice served as the Giomers group that was implanted in the subcutaneous tissue of right and left abdomen. Preparation of Nanocomposite and Giomers in the form of capsule with the size 2×1 mm in accordance with the manufacture instructions. The material was then placed into the subcutaneous tissue of mice abdomen using a pin set. The termination of mice treatment group and control group was made at a certain time interval, first 24 hours, 7th day, 14th day, 21st day, and 30th day after implantation of the materials in which 3 mice were being using.

Phase of histological preparation development being put in microscopic observation was divided into 2 phase; phase of subcutaneous histological preparation and development and phase of HE staining.

Production of subcutaneous histological preparation

Subcutaneous tissue was placed through which substances for restoration of Nanocomposite and Giomers content was established for lesion that received treatment of organ isolation. Further measures of subcutaneous histological preparation development were as follows: (1) Isolation of organ that was removed was in a condition ready to receive treatment of histological examination. Isolation of organ shattered was not used during study, since it will be disturbing the results of

diagnosis. Subcutaneous organ was put into a solution of formalin (10%) using spatula for 24 hours; (2) Put into a solution of serial alcohol 90, 90, 95% (as much as 2 times) and absolute alcohol; each for 1.5 hours; (3) Put into a solution of alcohol-xylol 1:1 and solution of pure xylol as much as 2 times. This process was called "buffer" or "clearing"; (4) Further process was called "infiltration" or serial paraffin as much as 3 times, each for 1.5 hours; (5) The entire process of dehydration, buffer and infiltration, was made in tissue processor; (6) Paraffin in an incubator was poured into a block of paraffin; (7) The process of embedding, the planting of organ in a block paraffin at room temperature for minimal of 24 hours, was made; it is later on stored in a refrigerator; (8) Block paraffin was sliced by microtome into 5 µm in thickness; (8) Slice of skin organ was immersed into water at room temperature and lukewarm water in a water bath; (9) The slice organ was attached to the object glass and dried on a heating plate; and (10) Preparations were ready to be dipped with a solution of Hematoxylin-Eosin.

Staining of hematoxylin-eosin

Measures taken in the staining of Hematoxylin-Eosin were follows: (1) Deparaffinization (removing paraffin from preparation) by putting preparation into solution of xylol as much as two times, each for 5 minutes; (2) Dehydration by serial alcohol starting with ethanol 95, 90, and 80% for 5 minutes; (3) Wash in running water; (4) Put into a solution of Hematoxylin for 5 minutes; (5) Wash in running water; (6) Immerse in a solution of Lithium Carbonate (0.5%) as much as 5 times; (7) Wash in running water; (8) Immerse in alcohol (80%) as much as 20 times; (9) Put into a solution of eosin for 1 minute; Wash in running water; (10) Dehydration by alcohol starting at 70,80,95% and ethanol; (11) Immerse in solution of Carbollic Xylol as much as 2 times; (12) Immerse in solution of pure Xylol as much as 2 times; and (13) Mounting (covering an organ by attaching a closing glass).

Method of dividing the mice

Thirty mice were divided in 2 groups. Group 1 consisted of 15 mice as a control group with Nanocomposite. Group 2 consisted of 15 mice which will be implanted with giomers in the subcutaneous tissue in abdomen region. The dividing of the mice

Table 1. Differences of inflammatory cell with Nanocomposite and Giomers based on Mann-Whitney test

	Lymphocytes	Neutrophil	Macrophages	Plasma Cell	Total
Mann-Whitney U	38.00	14.000	18.000	18.000	25.000
Wilcoxon W	53.000	479.000	483.000	483.000	490.000
Z	-1.750	-2.878	-2.689	-2.697	-2.358
Asymp. Sig. (2-tailed)	.080	.004	.007	.007	.018
Exact Sig. [2*(1-tailed sig)]	.086 ^a	.002 ^a	.005 ^a	.005 ^a	.016 ^a

into groups was done by using Federer's equation which is: $(t-1)(n-1) > 15$, where t was the amount of implantation ($=2$) and n was the repetition or replication. After using this equation therefore 3 mice will be replicated in the implantation group and even for the control group. These 3 mice will be taken out in every termination during the scheduled time for the research.

Implantation of the specimen

Before the implantation of the specimen was done, the skin in the abdomen region of the mice was shaved first. This was done so that the skin was seen as transparent this makes the implantation of the specimen easy. After that, the skin opened using a surgical knife and the specimen was inserted into the subcutaneous tissue in abdomen region using a pin set. The specimen that was mentioned here was the Nanocomposite and Giomers which is formed in an oval with the size of 2x1 mm. The mice were busied using ether inhalation. The opened skin was disinfected with Betadine®.

Data analysis

To observe tissue reaction of subcutaneous morphology by criteria as follows, increased in the number of inflammatory cells; lymphocyte, macrophage, neutrophil and plasma cell.

After production of histological preparation and coloration has been completed, the transformation of subcutaneous tissue is observed under

complexion microscope per visibility in the magnification of 50x, 100x (objective lens 10x and ocular lens 10x). The reaction of tissue can be observed by taking note of magnification at 400x and 1000x. One treatment group consists of 15 mice divided according to period of taking organ isolation at a certain time interval: 1st, 7th, 14th, 21st, and 28th days. Preparation was made for every mouse to be observed, each preparation area was observed from epidermis to randomly subcutaneous and inflammation cell is estimated and mean quality of percentage was put in statistic test. The control group consists of 15 mice through which the observation method was used equal to that of the treatment group.

RESULTS

In order to identify whether there was difference in the number of inflammatory cells (neutrophil, lymphocytes, macrophages and plasma cell) in the treatment group and control group, the statistic test used was Mann-Whitney. The result showed that there was no differences between treatment and control in Neutrophil, Macrophages, plasma cell and total.

The comparison of total sample with inflammatory cells, in order to identify the significance difference in the number of inflammatory cells (neutrophil, lymphocytes, macrophages and plasma cell) in the treatment group and control group, the statistic test used was Friedman.

Table 2. Differences of time period and the effects of changes in inflammatory cells based on Wilcoxon test statistics

	Giomers-Nanocomposites Day 1	Giomers-Nanocomposites 1 st week	Giomers-Nanocomposites 2 nd week	Giomers-Nanocomposites 3 rd week	Giomers-Nanocomposites 4 th Week
Z	-2.72 ^a	-1.604 ^b	-1.604 ^b	-1.604 ^b	-1.604 ^b
Asymp. Sig (2-tailed)	.785	.109	.109	.109	.109

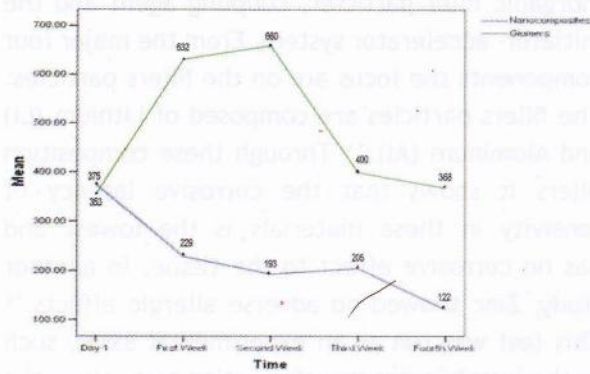


Figure 1. Differences of Inflammatory cells against time interval.

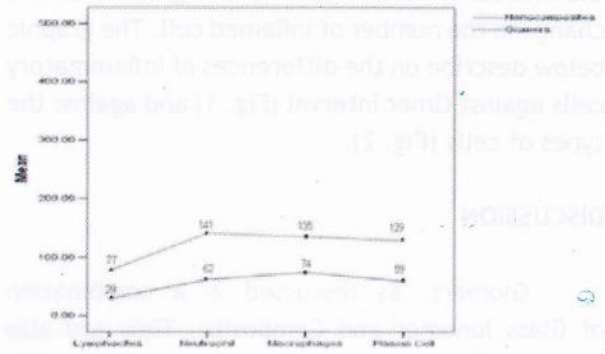


Figure 2. Differences of inflammatory cells against the types of cell.

Friedman test was used to test the significance of k related samples, originated from the same population, with ordinal minimal scale. The result showed that there was no difference in the number of inflamed cell in five time interval.

In order to identify whether there was difference in the number of certain inflammatory

cells (Neutrophil, Lymphocytes, Macrophages and Plasma Cell) in one time with other time in the treatment group, the statistic used Wilcoxon. The result showed that there is no difference of the effects caused by nanocomposite and gomers in each time interval. Or, it could be concluded that the differences of time interval doesn't influence

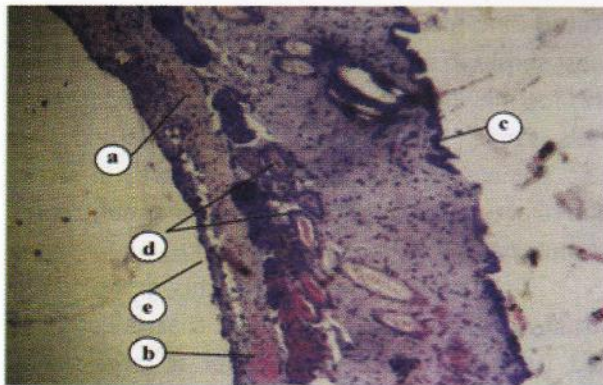


Figure 3. Histology of the subcutaneous tissue of mice for the control (HE staining, 100 x): (a,b) Collagen, (c) Epidermis, (d) Hairfollicle, (e) Adipose tissues.

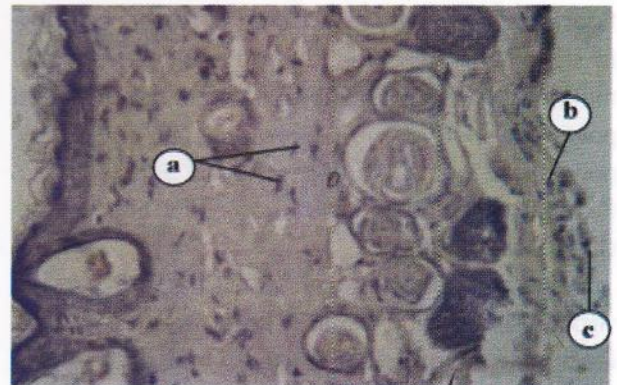


Figure 4. Histology of the subcutaneous tissue of mice for the control (HE Staining, 100 x): (a) Fibroblast, (b) Macrophage, (c) Lymphocytes.

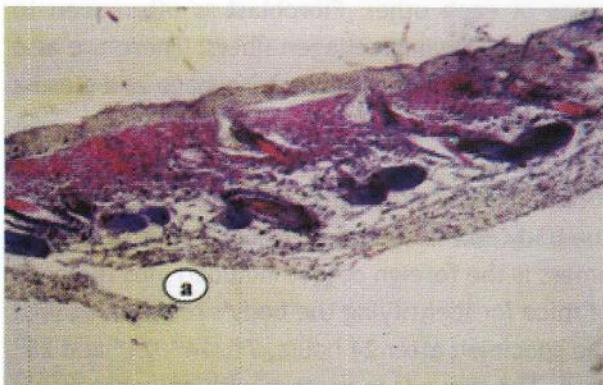


Figure 5. Histology figure of the subcutaneous tissue of mice after 24 hours (HE staining, 100 x): (a) Implantation of Nanocomposite.

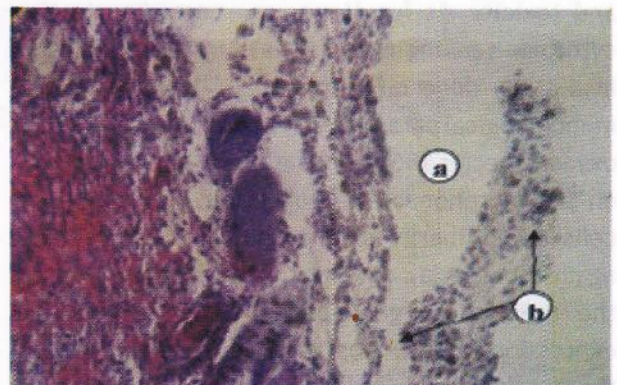


Figure 4 Accumulation of neutrophil cells (HE staining, 400 x): (a). Implantation of Nanocomposite, (b) Neutrophil cells.

the effects of nanocomposite and giomers on the change in the number of inflamed cell. The graphic below describe on the differences of inflammatory cells against timer interval (Fig. 1) and against the types of cells (Fig. 2).

DISCUSSION

Giomers, as discussed is a combination of Glass Ionomer and Composite. They are also known as Resin Modified Glass Ionomer.^{8,9} For this particularly type of material presumption amount of fluoride are released. When the amount of initial fluoride are released this explains by the nature of the fluoridated glass incorporated into the material and the extent to which the Glass Ionomer Matrix surrounds the glass filler in the material.^{8,9} Hence, it has a significantly thicker hydrogel layer which has been made by almost complete reaction with acid to form a sub structural glass ionomer matrix layer before incorporation in the resin matrix. This glass ionomer matrix contains much complexes fluoride and is easily penetrated by water resulting in significantly greater fluoride release from the material. Another possibility could be that the polymerization of the Giomer has not been fully polymerized. This could be applied and seen the instability of the inflammatory cells and in the time period (Fig. 3).

Glass Ionomers are supplied as powders of various shades and liquid. The powder is an ion-leachable aluminosilicate glass, and copolymers of acrylic acid. The material sets as a result of metallic salt bridges between Aluminum (Al) and Calcium (Ca).^{4,6} According to the cytotoxic ranking Calcium (Ca) did not show any sign of sensitivity and toxicity. As for Aluminum, according to the cytotoxic ranking is the lowest in the ranking.^{2,7} In a study done on Aluminum (Al) based on the immunological parameters it enhanced the reactive oxygen species (ROS). In this study it was focused on polymorphonuclear leukocytes (PMNs). These cells are primarily involved in the host defense against bacteria, example by the production of the reactive oxygen species (ROS). Thus, this expression of ROS was used as a parameter for the determination of interactive affects between several metal ions and PMNs.¹⁰

A resin composite is composed of four major components; they are organic polymer matrix,

inorganic filler particles, coupling agent and the initiator- accelerator system. From the major four components the focus are on the fillers particles. The fillers particles are composed of Lithium (Li) and Aluminum (Al).¹¹ Through these composition fillers it shows that the corrosive latency of sensitivity in these materials is the lowest and has no corrosive effect to the tissue. In another study, Zinc showed no adverse allergic effects.¹² This test was run as an experimental assay, such as the lymphocytes transformation test, along the dermal and intradermal. It was found that these ions had shown no adverse reactions.¹²⁻¹⁷

As a result from the analyzed statistics through the study above, this explains why Nanocomposite showed the lowest amount in inflammatory cells compored to Giomers (Fig. 5 and Fig. 6). Lithium according the cytotoxic ranking showed it had the lowest and the least toxicity. Barium (Ba), Zinc (Zn), Boron (B), Zirconium (Zr) and Yttrium (Y) ions have been used to produce radiopacity in the filler particle. From the ions stated Zinc has the rank in toxic potency on the most sensitive assay. The role played in Zinc is that this ion may induce gene activation in endothelial cells, similar to pro-inflammatory mediators. Zinc also stimulated leukotriene B4-release, in vitro, due to activation of polymorphonuclear neutrophil granulocytes (PMNs).¹⁵

The connective tissue in this group has a structure as a connective tissue that is compact and irregular. The collagen fiber is large-sized, typically it has been in the form of the combination of tick bundle. The type of fiber structure is called a compact or dense structure. In this tissue, the collagen fiber shows the same thickness among areas of each other. Fibroblast is often seen to be trapped among collagen fibers. There are also neutrophil, lymphocytes, macrophage and plasma cell.

Inflammation is a complex tissue reaction for eliminating a foreign object leading to lesion in the body. In this research, Nanocomposite and Giomers is the foreign object included into the body of mice for identifying the body's reaction against the specimen after 24 hours, 7th, 14th, 21st and 28th days. The tissue is in contact with an unbreakable material that is Nanocomposite and Giomers leading to chemical and mechanical irritation. The inflammation consists of two basic patterns acute

and chronic. In the acute inflammation, neutrophil is accumulated around the lesions - based tissues. In the chronic inflammation, it is followed by lymphocytes, macrophage and plasma cells such that it has been seen in the number of cells being increased in certain interval.

The subcutaneous tissue reaction within 24 hours after the implantation of Nanocomposite and Giomers, both materials showed the occurrence of the acute inflammation process. In addition, lymphocytes showed a significance differences in both control and treatment group.

Lymphocytes have a functional role which is related to immune reactions in defending against invading microorganisms. lymphocytes showed significant differences using Mann-Whitney test. These cell in meaning lymphocytes would be the severity parameter for inflammatory cells. Inflammatory cell which can be the severity parameter are the plasma cell. Plasma cells are derived from B lymphocytes and are responsible for the antibodies. Antibodies are immunoglobulin produced in response to penetration of antigens. Lymphocytes show whether the cell has underwent inflammatory reaction.

Neutrophil is an active phagocyte. This cell eats and destroys a foreign object. After doing its duty, neutrophil would then disappear. After 24 hours, this cell undergoes a cell death in apoptosis's, and then it is made to be phagocyte by macrophage along with the dead tissues (debris). During phagocytes process, both neutrophil and macrophage yield a product known as enzyme lysosom, being in uncontrolled concentration, it can lead to the destruction of tissues and chronic inflammation with the character of fibrosis tissue.

Macrophage has a duty for yielding a growth factor and cytokine stimulating the formation of new tissues after the tissues have been destructed in the acute inflammation process or called as a tissue repairing process. In this process, the destructed tissues are filled be fibro-vascular granulation tissues consisting of: new capillary tissues, proliferating fibroblast cells, and several macrophages. The process mentioned above has been seen in the histological description of mice's subcutaneous tissues determined for 7 days after the implantation. The collagen deposit that has been built in the tissue is a result of active synthesis of fibroblast cell. In the area around the place the

specimens has been implanted there is a collagen thickening. This collagen is increasingly thinned or disappeared completely in other areas. This shows that the body attempts to isolate a foreign object considered dangerous.

On the 7th day after the implantation of the specimen, the tissue showed a response of chronic inflammation. The lymphocyte infiltrating into the tissue was a sign that a chronic inflammation has occurred. The presence of the increase in number of lymphocytes cells on the 7th was far compared with that of the 24 hours. This was an indication that the acute inflammation process has been continued to be chronic. It was means that it was not only tissue repair but also tissue destruction is done continuously (Fig. 1 and 2). This can be concluded that Giomers showed an increasing amount of lymphocytes compared to Nanocomposite. This can be concluded that Nanocomposite had a better healing time interval and inflammatory cells compared to Giomers.

On the 14th day after the implantation, the tissue still showed a chronic inflammation response. The formation of granulation tissue, fibroblast proliferation, and fibrosis tissue, or called as fibrosis granulation tissue, it had been seen in the histological description of subcutaneous tissue in 14 days after implantation. The number of lymphocyte and macrophage cells on 14th day and 7th day were higher in Giomers. However, the number of cells was lower than that of cells in control group. Possibly the lymphocyte was less active in establishing antibodies such that reducing the macrophage that was active in phagocytosis as compared with on the 7th day. Consequently, enzyme lysosom yielded by macrophage was also in minimum concentration, and the tissue destruction was not as tremendous as on the 1st day and 7th days.

Possibility, the chronic inflammation process was continued until the foreign object was, in this case Giomers and Nanocomposite eliminated. The formation of collagen rasp tissue is a tissue healing process because regeneration does not occur. Consequently, the tissue established by this collagen will not run its function as like properly normal tissue or even it can be lost in its function.^{3,10,18}

The chronic inflammation response was continued up to 21st day and 28th day after the

implantation. This can be concluded that based on the graphic shown, Nanocomposite showed a decrement in time interval compared to Giomers. The histological description showed that there are granulation tissue, fibrosis tissue, and collagen deposit that is very thick or called as collagen rasp tissue. The increase of collagen thickness and granulation tissue occurred on 21st day until 28th day.

CONCLUSION

Both of the implantation led to a tissue reaction in a form of inflammation viewed on 7th day, 14th day, 21st day and 28th day after Nanocomposite and Giomers were implanted but through the analysis of statistics it did not show significant differences in rapid inflammatory reaction. The implantation of Nanocomposite and Giomers in mice's subcutaneous tissue showed a significance differences in lymphocytes in both treatment and control group. The number of inflammatory cells (neutrophil, lymphocytes, macrophages and plasma cell) in five time interval in both Nanocomposite and Giomers did not show significant differences in statistics analysis. With the analysis of statistics, the difference of time interval does not influence the effects of Nanocomposite and Giomers on the changes of inflammatory cells (neutrophil, lymphocytes, macrophages and plasma cell).

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