Comparison of the Total amount of Macrophages on Full Thickness Wound Bed in The Use of Tulle, Freeze-Dried Amnion, and Microbial Cellulose

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Abstract

Background: The wound is the most frequent problems faced by plastic surgeon. Many factors can affect the wound healing process. Macrophages are as one indicator of wound healing which present in the wound within 24-48 hours after injury. Currently, there is a variety of wound dressing available which can increase the levels of macrophages in the wound healing process according to previous research. **Objectives:** To compare the total amount of macrophages on full thickness wound bed in the use of tulle, freeze-dried amnion, and microbial cellulose on the second day. **Material and Method:** The study design was experimental, post-test only group design using 21 male rats *Rattus norvegicus*. The wound was closed with tulle, amnion, and microbial cellulose which was evaluated on the second day. The samples wounds were fixed by 10% formalin solution then examination of samples was conducted by Wright-Giemsa staining routine/Hemato-eosin under a microscope. **Results:** Macrophages obtained at a given tulle ranges between 41-96, freeze-dried amniotic at 51-142, and the microbial cellulose at 55-96. In other hand, the mean number of macrophages in the wound by the microbial cellulose at 77.4; its 1.3 times higher than the given tulle at 59; meanwhile its 0.9 times lower than the freeze-dried amniotic given by 83. **Conclusions:** There were no differences in the increase number of macrophages in the wound bed by the use of tulle, freeze-dried amnion and microbial cellulose on the second day.

Keywords: *Amnion, macrophage, microbial cellulose, tulle, wound healing*

Introduction

The wound is the most frequent problems faced by a plastic surgeon that both injuries caused by trauma, systemic disorders, or injuries resulting from surgery. It is a break or discontinuity of the integrity of the skin, mucosa, or tissue. The process of wound healing occurs in three phases, there are inflammatory phase, proliferation phase, and remodeling phase. Many factors can interfere the healing process that lead into increasing the length of healing time, morbidity and mortality of patients, which resulted in a bad appearance of the wound, aesthetically [1].

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Although the etiology of an injury could be different, the healing process still remains the same. Damage to the tissue will stimulate the activation of extrinsic and intrinsic factors, acute and chronic inflammatory responses, the process of neovascularization through angiogenesis and vasculogenesis, cell proliferation, mitosis, apoptosis, and extracellular matrix deposit and remodel the matrix [2].

In addition to the intracellular processes, wound healing could be affected by the condition of the wound. The condition of the wound made in such way to create the appropriate atmosphere to accelerate the wound healing process. Macrophages are very important in wound healing process since they are capable of releasing cytokines and materials. They are needed to help in the healing process of wound. Macrophages present in the wound within 24-48 hours after injury

and peaked at 48-72 hours. Moreover, macrophages are capable of producing growth factors, such as TGF-β and an epidermal growth factor that work in regulating the inflammatory response, stimulate angiogenesis, and granulation tissue formation realigned [1,3].

Macrophages play an important role as the main source of most of the active ingredient in the process of wound healing. The majority of the necessary growth factors in wound healing are produced by macrophages. Macrophages are required in the process of wound healing due to the removal of macrophages. If there is no macrophage, so it will cause wound healing to stop [4,5].

The process of wound healing occurs in three phases, namely the inflammatory phase, proliferation phase, and remodeling phase. After the injury, blood vessels constrict and retraction broke with hemostasis reaction. Furthermore, the release of histamine from mast cells that also works to increase vasodilation and capillary permeability. Inflammatory phase is characterized by increasing of vascular permeability, especially for leukocytes, neutrophils, and macrophages [1,6].

Currently, there are a variety of dressings available which according to research can increase the levels of macrophages in the wound healing process. In this study, researchers tried to observe the local response in the wound bed with tulle applications, freeze-dried amnion, and microbial cellulose associated with macrophages as one of the main components in the wound healing. It is expected that the results of the research can be used as the development of the use of freeze-dried amnion and microbial cellulose.

Materials and Method

This research uses experimental study design, post-test only group design. Operational variables consist of microbial cellulose, freeze-dried amnion, and tulle [7]. We use Cuticell® epigraft as microbial cellulose, is a layer of pure cellulose derived from *Acetobacter xylinum* bacteria that has a great potential in wound healing. Microbial cellulose is proven to increase the rate of wound epithelialization. We used freeze-dried amnion which has gone through the process of freeze-dried and sterilization with gamma rays from Tissue Bank of Dr. Soetomo Hospital. Meanwhile, we used Cuticell^{R} Classic as a tulle without any other additional ingredients (antibiotics).

Three months old of 21 mice (*Rattus novergicus*) were selected in this study which was randomized using providing labels. Mice were grouped into three groups consist of seven mice; tulle is applied in group 1, freeze-dried amnionis applied in group 2, while microbial cellulose is applied in group 3. Mice injected with ketamine 20mg/kg intra-muscular. Each mouse was purposely being cut in 1x1cm square-shaped size on their backs that will be disinfected with Betadine 10% and savlon 1: 30. Operation field was narrowed by dock sterile then a full-thickness wound was made by tangential excision using blade number 15. Injury in the group 1 covered with tulle, wound in the group 2 covered by the freeze-dried amnion, while in the group 3, the wound covered with microbial cellulose. After that, the wound was treated in a closed wearing thick sterile gauze and covered by stitches on the back. All mice were given the intramuscular injection of Penicillin Procaine 100mg/kg. Mice kept in different cages and fed by the same type and amount.

The wound was evaluated on the second day, where ten mice were sacrificed by injecting phenobarbital 60-100 mg/kg intraperitoneal on lateral midline area between processes hypoid and pubis. The second day was cones because macrophages infiltration in the wound bed started at 48-72 hours post-injury [2]. Studies of macrophage activation showed that to look at the ability of macrophage activation should use the fastest time limit [4]. Based on the data, the authors chose the fastest time which was the second day after the injury. On histologic examination, the wound dimensions of approximately 0.5 cm outside the wound edges was included as healthy tissue. Then, the tissue was removed until the muscle layer that will be folded with filter paper and fixed with 10% formalin solution**.** Routine examination was performed by Wright-Giemsa staining/ Hematoksilin-eosin, then viewed under a microscope to count the number of macrophages with the help of graticule lens (ischak wirjatmadi).

Results

Normality test using the Kolmogorov-Smirnov test was performed on the number of macrophages before being tested statistically. The result of normality test for the three groups showed p value >0,05 which means all data were normally distributed.

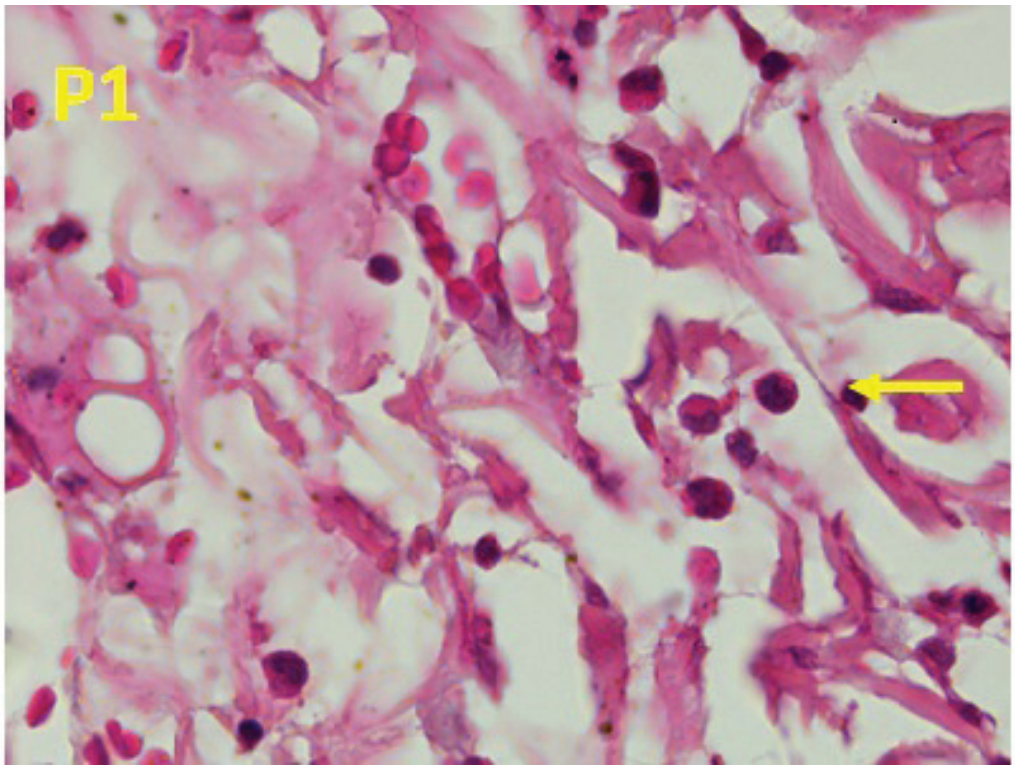

Figure 1. The number of macrophages in the healing tissue. Macrophage cells (arrows) on the injury area with treatment using tulle (HE staining. Magnification 100x; H600L Nikon microscope; Fi2 300 Megapixel Camera DS).

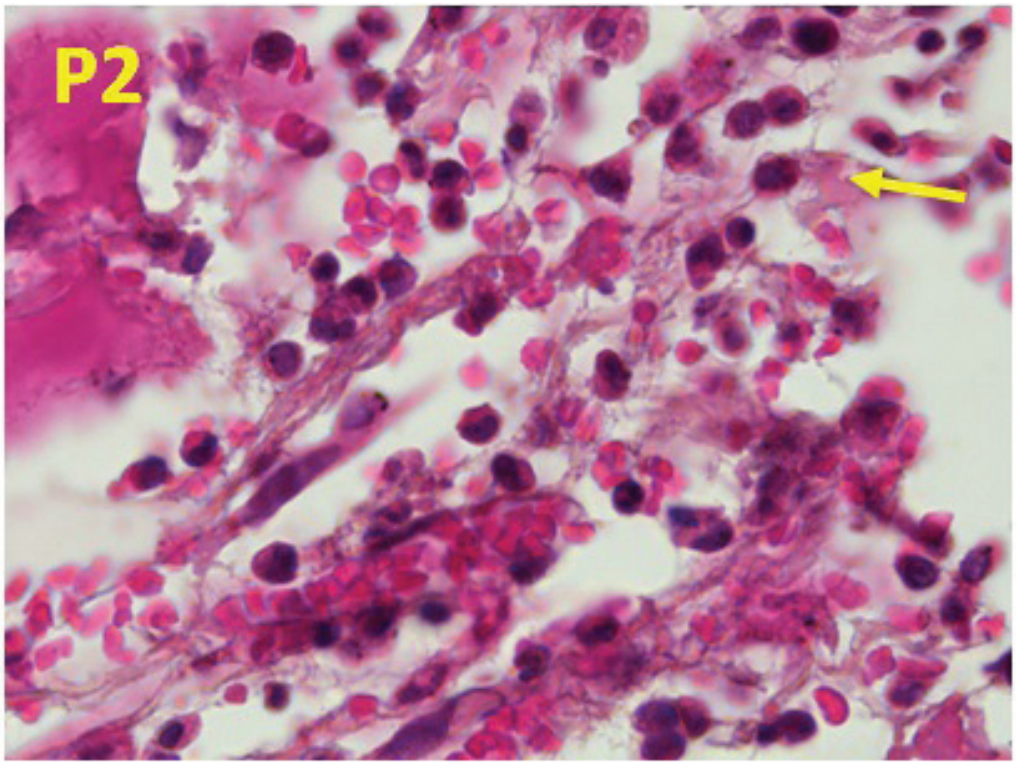

Figure 2. The number of macrophages in the healing tissue. Macrophage cells (arrows) on the injury area with treatment using freeze-dried amnion (HE staining. Magnification 100x; H600L Nikon microscope; Fi2 300 Megapixel Camera DS).

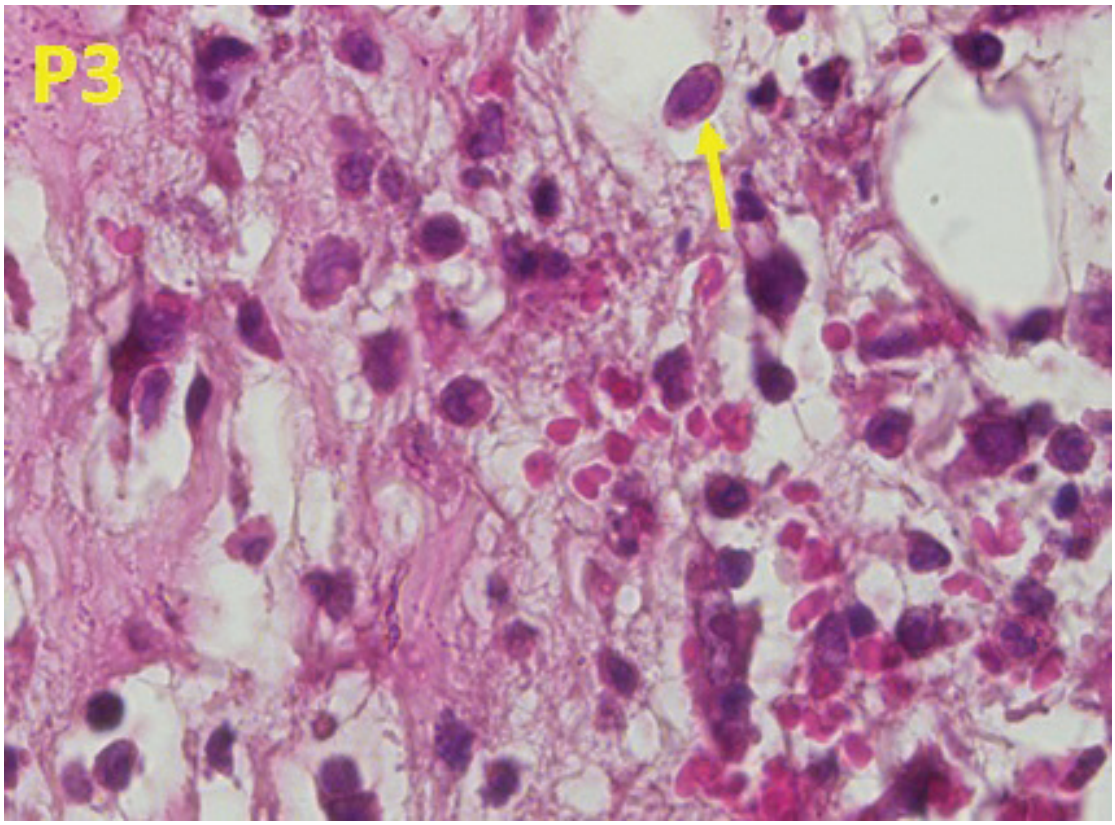

Figure 3. The number of macrophages in the healing tissue. Macrophage cells (arrows) on the injury area with treatment using microbial cellulose (HE staining. Magnification 100x; H600L Nikon microscope; Fi2 300 Megapixel Camera DS).

Table 1. The amount of macrophages cell in each treatment

Treatments	Mean	SD	Minimum	Maximum
Tulle	59.9	24.7	41	96
Freeze-dried amnion	83.0	32.9	51	142
Microbial cellulose	77.4	15.2	55	96

It can be seen in figure 1, 2 and 3 that the number of macrophages in the healing tissue was observed histologically using 100x magnification. The calculation of macrophages was obtained by the treatment result using tulle that was about 599 by the maximum amount of 96 macrophages cells. The treatment of microbial cellulose showed the mean of 77.4 by the maximum cell of macrophages as same as tulle treatment which was about 96 cells. After that, the treatment used freeze-dried amnion resulted mean of 83.0 by maximum amount of macrophages cell was 142. Therefore, the treatment used freeze-drive amnion resulted effectively.

However, the standard deviation (SD) did not show the difference significantly and can be seen in figure 4. Macrophage of scar tissue is obtained at a certain range, for tulle at 41-96, freeze-dried amniotic at 51-142 and the microbial cellulose at 55-96.

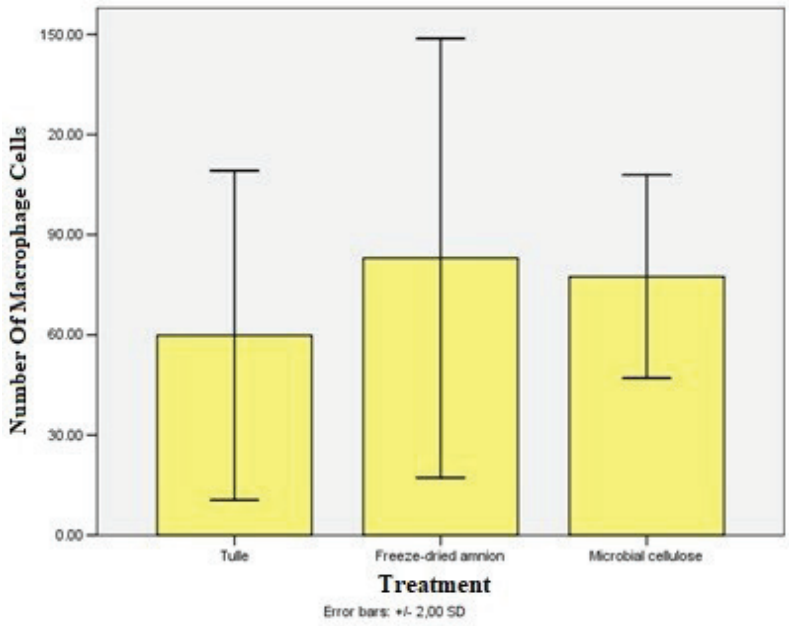

Figure 4. Average number of macrophage cells per treatment.

It can be seen in table 1 that the mean number of macrophages on the wound by the microbial cellulose was 77,4 which means that it was 1.3 times higher than the tulle at 59; meanwhile it was 0,9 times lower than the freeze-dried amniotic at 83. Statistical test results obtained using ANOVA with p value >0.05 which means that there was no significant difference in the number of macrophages in the provision of microbial cellulose, tulle, and freeze-dried amniotic.

Discussion

In the previous studies stated that the amnion has an impact on the local immune system by giving more macrophage expression and regulate the function of macrophages [8,9]. In addition, the amnion gives more influence number of macrophages in bed sores than tulle. The amnion increases the number of macrophages 2.5 times more than tulle. Microbial cellulose dressing may accelerate epithelialization while bacterial cellulose can regulate inflammation by giving effects on the formation of macrophages and lymphocytes [10,11]. Those studies are different with the results of this research.

The macrophage acts as an indicator of the wound healing process that is absolutely necessary for the wound healing process. Macrophages are needed in the wound healing since they are capable of producing cytokines and growth factors such as TGF-β and the epidermal growth factor that works in regulating the inflammatory response, stimulate angiogenesis, and granulation tissue formation realigned. Macrophages present in the wound within 24-48 hours after the injury and peaked at 48-72 hours.

Other studies claimed that the use of amnion on wound gives less significant result in influencing the inflammatory phase of the healing process. Despite the significant increase seen in the process of angiogenesis in the granulation phase, it does not affect the process epithelialization, fibroplasia or fibrosis in phase significantly [12].

One of the factors that play a role and always used in the wound treatment is wound dressings (bandages). The amnion contains mesenchymal stem cells and growth factors that can accelerate the healing of a wound [13]. The amnion effects on the local immune system by giving more macrophage expression and regulate the function of macrophages [8,9]. Microbial cellulose dressing can accelerate epithelialization rate [10]. Bacterial cellulose can regulate inflammation that effects on the formation of macrophage and lymphocyte [11,14]. Bacterial cellulose stimulates macrophages to produce IL-12, p40, and TNF-α which play a role in anti-inflammatory and antigenic processes [15]. This study proves that there is no difference in the number of macrophages in the wound bed given tulle, freeze-dried amnion, and microbial

cellulose.

Conclusions

There was no difference in the number of macrophages in the wound bed given tulle and microbial cellulose, given freeze-dried amnion and microbial cellulose on the second day.

Ethical Clearance: This study protocol was approved by ethical clearance Dr.Soetomo Teaching Hospital Surabaya, Indonesia.

Conflict of Interest: The author reports no conflict of interest of this work.

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