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Development of made coral sponge as a haemostasis agent for acceleration of postoperative wound healing

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Abstract:

Surgical injury is a trauma that is caused after doing surgery on the human body. The process of healing post-operative wounds requires a long time. There are phases of wound healing that must be passed until the wound heals. One important step that needs to be done is to control bleeding after surgery. Most bleeding controls that are used still have limitations, namely the nature that is not biodegradable so it cannot be absorbed by the body. General measures to stop bleeding generally use pressure or ligation or non-absorbable media. However, non-biodegradable agents in their use must be taken back after the wound heals which can trigger new bleeding. Calcium carbonate contains a high calcium element per unit weight. In addition calcium carbonate produces anti-inflammatory activity in acute and subacute inflammation, as well as a biocompatible and biodegradable material. Wound healing can be characterized by collagen density, the number of new blood vessels (angiogenesis), and the number of neutrophils. The purpose of this study was to determine whether artificial coral sponges containing calcium carbonate (CaCO3) have good haemostasis ability (characterized by an increase in the number of new blood vessels), a controlled level of inflammation (indicated by the number of neutrophils), and collagen density. Sixteen Sprague Dawley mice were divided into 4 groups with 4 mice in each group. Each rat was injured four times with a wound size of 1x1 cm^2. In three wounds different composition of the sponge was applied, namely one wound with 25% coral sponge, two wounds with 33% CaCO3 coral sponge, three wounds with gelatin-only sponge, and four wounds with gauze (control group). The tissue was taken on days 1, 4, 7, and 12 and histological preparations were stained with HE (Hematoxylin Eosin) and Mallory staining. After histology preparations were obtained, observations were made using a binocular microscope with a magnification of 4x100 objective lenses. Clinical observations showed that the wound with a CaCO3 coral sponge showed a more complete wound closure. (HISTORY RESULTS). Therefore overall, the results of this study indicate that there is an influence on the use of artificial coral sponges for accelerating postoperative wound healing.

Keywords: wound healing, artificial coral sponge, calcium carbonate, collagen density, number of blood vessels, and number of PMN.

Preliminary:

The process of wound healing goes through several phases. The first phase is the inflammatory response. In this phase, removal or destruction of dead tissue occurs and prevention of infection is characterized by an increase in vascular permeability by thrombin after haemostasis. Neutrophils have an important role as the first signal sender when cellular damage occurs and also sends chemotaxis signals to the fibrin patch. Next vasodilatation of nearby blood vessels occurs and there are many neutrophils in the wound area stimulated by Interleukin (IL) -1, Tumor Necrosis Factor (TNF) -α, Platelet Factor (PF) -4, Transforming Growth Factor (TGF) -β, Platelet Derived Growth Factor (PDGF), and bacterial products. Polymorphonuclear leukocytes (PMN) begin to clear invading bacteria and cellular debris. Monocytes will go

to the wound area and turn into macrophages for 48 to 72-96 hours after the injury. Macrophages are responsible for phagocyte debris and bacteria. Growth Factor production is needed to produce extracellular matrix by fibroblasts and new blood vessels. Activated macrophages are the key to the proliferation phase because they will mediate angiogenesis, fibroplasias, and nitric oxide synthesis. The second phase is the proliferation phase. This phase is characterized by granulation tissue in the wound consisting of new capillary nets, fibroblasts, and macrophages. In addition to granulation tissue formation, epithelialization is also a major process in wound healing. Angiogenesis is characterized by endothelial migration and capillary formation. New blood vessels appear in the collagen matrix formed by fibroblasts. Fibroblasts migrate from the surrounding tissue to the wound and start to collagen synthesis and proliferate. Clinically, wound contraction is a natural response from the body to localize the wound and make the wound area narrower. The third phase is the maturation phase. This phase is the last and longest phase in wound healing which involves remodeling granulation tissue. This phase takes 8-21 days after the injury or it may take longer. The main characteristic of the maturation phase is the regular deposition of collagen which causes remodeling of collagen and scar tissue contraction. Movement of fibroblasts attracts collagen fibers for scar tissue to contract. The final phase is also marked by a balance of collagen deposition and degradation which results in normal scarring.

Surgical injury is a trauma that is caused after doing surgery on the human body. The process of healing post-operative wounds requires a long time. There are phases of wound healing that must be passed until the wound heals. One important step that needs to be done is to control bleeding after surgery. Most bleeding controls that are used still have limitations that are non-biodegradable traits that cannot be absorbed by the body. Many studies have been conducted which aim to speed up the process of healing post-operative wounds. According to Tsugawa et al. calcium carbonate contains a high Ca element per unit weight. According to Karnad et al. calcium carbonate produces anti-inflammatory activity in acute and subacute inflammation. Theory according to Vergaro et al. calcium carbonate is a biocompatible material. Besides that the theory put forward by Ueno et al. calcium carbonate is a biodegradable material. Based on these theories, it can be seen that the content in calcium carbonate (CaCO3) is naturally well applied in the human body without causing adverse side effects.

Haemostasis agents used so far are conventional haemostasis agents and biodegradable haemostasis agents. According to Gu et al. examples of conventional haemostasis agents are ligation or pressure. Supported by Rupali et al. Conventional homeostasis agents are non-biodegradable agents which have limited closure of wounds and cause bleeding. For biodegradable haemostasis agents such as pig or cow gelatin where the agent has adverse side effects and toxicity due to its volume and ability to induce infection. On the other hand, according to Said et al, gelatin on the Indonesian market is still dominated by gelatin originating from abroad or import. Through a number of deficiencies in haemostasis agents, biodegradable materials are needed that are effective and convenient to use. Innovation of artificial coral sponges containing calcium carbonate (CaCO3) with porous structures can be an alternative agent used to accelerate the wound healing process. In the process of wound healing required haemostasis agent where the formulation of haemostasis agent can be found in the form of foam or sponge (Rupali, et al,). The advantage of this artificial coral sponge is as an alternative media that is biodegradable so that it can be easily absorbed by the body without causing adverse effects and can be produced by yourself from natural materials that can be found in Indonesia. Provision of medical devices which according to the latest data from the Ministry of Health of the Republic of Indonesia must still be imported from abroad, which amounts to 97.2%. By researching the development of artificial coral sponges as a haemostasis agent to accelerate wound healing, it is hoped that Indonesia can produce

its own alternative material as a haemostasis agent from natural materials native to Indonesia.

Berdasarkan pertimbangan material yang biodegradable dan material tersebut yang mudah ditemukan dalam negeri, peneliti berinovasi memanfaatkan spons koral buatan yang mengandung kalsium karbonat (CaCO3) sebagai agen haemostasis yang dapat mempercepat proses penyembuhan luka pasca operasi.

Based on this explanation, research is needed to examine the effect of artificial coral sponges as haemostasis agents for accelerating postoperative wound healing. The formulation of the problem in this study is whether artificial coral sponges containing calcium carbonate (CaCO3) can act as haemostasis agents for accelerating postoperative wound healing? The purpose of this study was to determine whether artificial coral sponges with calcium carbonate (CaCO3) content could act as haemostasis agents for accelerating postoperative wound healing.

Research Methods:

Making Artificial Coral Sponges:

After getting the Ethichal Clearance from the Ethics and Advocacy Commission of the UGM Faculty of Dentistry, we made artificial coral sponges at PT. Swayasa Prakarsa Yogyakarta. The first sponge with the gelatin ratio formula: calcium carbonate = 4: 1; the second sponge with the gelatin ratio formula: calcium carbonate = 3.75: 1.25; third sponge with a formula ratio of 7.5 grams of gelatin gelatin in 100 ml of water Made an artificial coral sponge with a concentration of 25%, 33%, and a sponge with a gelatin content of 7.5 grams. The coral sponge contains calcium carbonate and gelatin. The next step is gelatin weighing 3.75 g, 4 g and 7.5 g, respectively, and put into 100 mL of distilled water in different beaker glasses. After that the next stage is swelling for about 30 minutes. Then the beaker with the contents of gelatin of different weights is placed on the magnetic stirrer for approximately 30 minutes until the gelatin is dissolved. Next is the addition of calcium carbonate (CaCO3) weighing 1.25 g in beaker glass 3.75 g gelatin and calcium carbonate (CaCO3) weighing 1 g in beaker glass 4 g gelatin. The beaker is closed and then placed on a magnetic stirrer and rotated for about 2 hours until dissolved. The next step is to measure pH (the pH obtained is 7.4). After measuring the pH the overhead stirrer is carried out for about 2 minutes to get foam. Foam is then poured into a mold and then put in the freezer for 24 hours. The next step is freeze drying for 24 hours. After freeze drying, DHT is done for 48 hours. The last step is sterilizing the sponge stored in aluminum foil and sterilized at the Bethesda Hospital in Yogyakarta and ready for use.

Treatment of experimental animals:

16 Sprague Dawley rats aged 4-5 months with a weight of 250-300 grams male sex were divided into 4 groups with six repetitions. Each rat was injured with a size of 1x1 cm^2 on the back of 4 wounds. Wound 1 is upper left back, wound 2 in upper right back, wound 3 in lower left back, and wound 4 in right lower back. In each mouse given a sponge application that has been made plus control in the form of sterile cotton.

Making histopathological preparations:

Clinical observation of wounds and tissue retrieval was carried out on days 1, 4, 7, and 12. Animals were tried to be eusthanated by anesthetized with ketamine until overdosed and then euthanated with cervical dislocation. The wound is photographed and measured, after which the tissue is removed and stored in a preparatory bottle containing 10% formalin buffer to maintain the cell structure. Then sent to the Anatomical Pathology Laboratory to make histological preparations by staining HE (Hematoxylin Eosin) and Malori.

Conclusion:

Wounds that are more perfect than wounds that are not given an artificial sponge There is a difference in the clinical appearance of wounds that are given an artificial sponge with a wound without being given an artificial sponge. Clinical results show that wounds

applied by artificial coral sponges show wasting.