SURAT PERNYATAAN

Saya yang bertanda tangan dibawah ini:

Nama: Dr. Rima Parwati Sari, drg., MS.Kes
NIDN: 0001047302

Menyatakan bahwa karya ilmiah dengan judul "Granule effectiveness of Anadara granosa shell–Stichopus hermanni to the number of blood vessels on 14th day after tooth extraction" yang dilaksanakan di Surabaya pada tanggal 29-30 November 2019 dinyatakan asli.

Demikian surat pernyataan ini saya buat, atas perhatiannya saya ucapkan terima kasih.

Surabaya, 18 November 2020

Mengetahui ketua LPPM

Yang membuat pernyataan

Dr. Rima Parwati Sari, drg., MS.Kes
PROCEEDING BOOK
DENTISPHERE
INTERNATIONAL SCIENTIFIC MEETING
5TH ASEAN PLUS & TOKUSHIMA
JOINT INTERNATIONAL CONFERENCE

"Beyond Dentistry: Synchronizing Science, Technology and Technical Skill in Modern Dentistry"

NOVEMBER 29-30th, 2019
Shangri-La Hotel, Surabaya
WELCOME NOTES

DEAN
FACULTY OF DENTISTRY
UNIVERSITAS HANG TUAH
SURABAYA

Dear Colleagues,

It’s great honour to welcome you to Surabaya and to Dentisphere 4th International Scientific Meeting in collaboration with 5th ASEAN Plus & Tokushima Joint International Conference which is held on 29-30th November, 2019 at Shangri-La Hotel Surabaya, Indonesia. My great appreciation to all the speakers from Japan, Korea, Malaysia, India and Indonesia, thank you for the contribution and participation and your willingness to come and share the valuable knowledge and experience. It’s been honour to us that this forum may be a part of strong role as quality control mechanism to ensure sustainability and continuous improvement of dentist.

The theme of this Dentisphere 4th is “Beyond Dentistry: Synchronizing Science, Technology and Technical Skill in Modern Dentistry” This is addressed to meet our aims to provide our nation a generation of professional and skilful dentist with continuously update knowledge. We hope this event will be increase our professionalism to all dentist and participants.

My appreciation to the committee, for arranging this event very well. Hope the seminar will be well done accomplished tomorrow. Also, I would like to thank all sponsors who support this event. For the speaker, thank you for the contribution support of the seminar. And for all the participants, thank you for joining the Dentisphere 4th, please enjoy the seminar and the events. I would like to ask for apologize if maybe in some ways we have some limitation in serving you on the event. Finally, I hope we all could get the benefit and advantage from this seminar to raise our professionalism in dentistry, in each of our ways.

Sincere regards,

Lita Agustia, drg., M.HKes
Dean Faculty of Dentistry, Universitas Hang Tuah

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Dear Colleagues,

It is a great pleasure for us to be the organizer of the Dentisphere 4th International Scientific Meeting in collaboration with 5th ASEAN Plus & Tokushima Joint International Conference by Faculty of Dentistry, Universitas Hang Tuah Surabaya, Indonesia. We extend our warmest welcome to all Participants, Speakers, and Sponsors that make this Dentisphere 4th to be a successful conference.

Under the theme of "Beyond Dentistry: Synchronizing Science, Technology, and Technical Skill in Modern Dentistry", this meeting will offer a platform to learn and exchange ideas with a host of internationally and national speakers. Dentisphere 4th will provide participants with unique opportunities to develop their professional knowledge and skills as well as to network with another audience. I also strongly encourage you to take advantages of the presence of dental companies to keep up to date with evolving technologies of equipment and the latest dental materials. We do hope that this seminar will allow all participants to capitalize enough knowledge and experience keep in touch with issues worldwide in dental health.

Lastly I wish to thank all Participants, Distinguish Speakers, Sponsors and all who contribute for the success of the Dentisphere 4th in Surabaya. Hope you not only have an event for developing our professionalism, but also you could enjoy a nice stay and have a memorable excursion on Surabaya. Thank you for your kind attention, have a nice, enjoy and fruitful discussion and God Bless You.

Sincere regards,

Vivin Ariestania, drg, Sp.Pros
Chairperson of Dentisphere 4
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PP.1 Granule effectiveness of Anadara granosa shell-Stichopus hermanni to the number of blood vessels on 14th day after tooth extraction
Hansen Kurniawan, Rima Parwati Sari
Granule Effectiveness Of Anadara Granosa Shell–Stichopus Hermanni To The Number Of Blood Vessels On 14th Day After Tooth Extraction

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Abstract

Background: Hemostasis is the key to the healing process after tooth extraction. Disorder of hemostasis can cause disturbed angiogenesis which results in delayed healing of sockets and alveolar bone resorption. Biphasic calcium phosphate (BCP) is a combination of duo calcium phosphate which can be synthesized from the shell of anadara granosa which functions as bone substitute material. Stichopus hermanni contains hyaluronic acid which can stimulate angiogenesis which plays a role in the socket healing process. Purpose. The aims of this study to determine the granule effectiveness of Anadara granosa shell-Stichopus hermanni to the number of blood vessel on 14th days after tooth extraction. Methods. Twenty four male Wistar rats were divided into four groups. Lower left incisor was extracted, then given granule scaffold from Anadara granosa shell as group control (AG), treatment group was administered with granule scaffold from Anadara granosa shell-Stichopus hermanni with concentration of 0.4%-0.8%-1.6% (AGSH1-AGSH2-AGSH3). We have been developed a BCP synthesized from anadara granosa combined with stichopus hermanni extract for granule scaffolds using freeze-dried method. The socket was left up to 14 days after tooth extraction immediately. Data were analyzed with ANOVA and LSD tests (p<0.05). Results. Statistical analyses showed there to be significant differences on the number of blood vessel between AG (6.5±1.94) and AGSH2 (13.17±2.56) – AGSH3 (10.33±3.27), AGSH1 (8.55±2.74) and AGSH2, but between AG-AGSH1, AGSH3-AGSH1 and AGSH2-AGSH3 no significant differences. Conclusions. Anadara granosa shell-Stichopus hermanni 0.8% is the most effective granule to increase the number of blood vessels on the 14th day after extraction.

Keywords: Anadara granosa shell; Stichopus hermanni; tooth extraction, blood vessel, 14th day

INTRODUCTION

Tooth extraction is a treatment by dentists in hospitals, clinics, and private practice. Tooth extraction is a surgery that involves bone (alveolar bone) and soft tissue (periodontal ligament and gingiva) in oral cavity (Nugroho, 2012; Dharmawan et al., 2013). Post-extraction wounds can easily heal but alveolar bone defects cannot heal completely, sometimes various kinds of complications such as dry socket, acute alveolar infection, acute alveolar inflammation which will slow the healing process (Dharmawan et al., 2013; Kareem 2008). Several factors such as lack of blood supply, the presence of foreign bodies, prolonged inflammatory processes, disruption of
the formation of fibroblasts and collagen fibers can inhibit healing in tooth sockets (Dharmawan et al., 2013; Sahir, 2012).

Angiogenesis or the formation of new blood vessels is very important in the healing process of the socket because blood vessels supply oxygen and nutrients that are important to support cell metabolism (Mitchell et al., 2009; Tonnesen, 2000). Angiogenesis plays a direct role in maintaining the function of various tissues and organs (Frisca et al, 2009). The process of angiogenesis is divided into several stages starting from the initiation process, namely the release of the protease enzyme from activated endothelial cells; vascular blood vessel formation, including extracellular matrix degradation (Extra Cellular Matrix, ECM), migration and proliferation of endothelial cells, and the creation of new ECMS, which are then followed by the maturation / stabilization of blood vessels that are controlled and modulated to supply tissue requirements (Frisca et al., 2009).

Some researchers argue that the use of drugs after tooth extraction can reduce the possibility of complications and reduce excessive bone resorption by accelerating the wound healing process (Dharmawan et al., 2013; Heerden, 2012).

Blood clams (Anadara granosa) have many uses, one of which is that they can be processed as food, so that the shells of blood clams (Anadara granosa) which are leftovers will cause considerable waste. Utilization of blood clam shells (Anadara granosa) is still small, so it can be used as a basis for bone graft research and can help with waste shellfish treatment (Afranita, 2014). This blood shell skin is osteoconduction and has a mineral composition of CaCO3 98.7%, Mg 0.47%, Na 0.91%, P 0.018%, K 0.039%, others 0.19% (Fe, Cu, Ni, B, Zn and Si) (Awang, 2007). In Hafisko's research, after going through a series of synthesis processes using the precipitation method, HA-TCP content was obtained. In addition to precipitation, the hydrothermal method is an alternative in HA synthesis of calcium carbonate compounds (Istifarah et al, 2017).

The process of healing bone damage will be faster by adding other components of material that are osteoinduction, this material can be obtained from marine life, one of which is sea cucumbers. The nature of osteoinduction will change the cells in the graft into osteoblast cells that form bone. Sea cucumbers are animals that have high economic and nutritional value, besides that, sea cucumbers have bioactive content, such as hyaluronic acid (HA). Based on research conducted by Dahiy, and Kamal (2013), the use of HA with a concentration of 0.8% states that HA accelerates bone regeneration by chemotaxis, proliferation and differentiation of mesenchymal cells. Topical administration of HA in extraction of dental animals showed a closure of the socket and in terms of histology there was an increase in bone formation activity compared to the control group (Demarosi et al., 2007).

Granule is a form of scaffold that has a small size, but it is porous which gives room for cells to grow and mature (Laurencin, 2008). Granule is needed in the manufacture of polymers that act as a binder which will later go through the freeze drying process and produce granule formations (Kang, 2014).

Based on this study, research is needed to determine the effectiveness of granules from a combination of shells of blood shells (Anadara granosa) and golden sea cucumbers (Stichopus hermanni) on increasing the number of blood vessels in the socket after tooth extraction on the 14th day with a concentration of Stichopus hermanni by 0.4%, 0.8% and 1.6%.

MATERIAL AND METHODS

This research is true experimental using randomization in sample grouping. Preparation of the study began with the making of a graft from Anadara granosa shell (AG) which was synthesized into the HAp through a calcination process, hydrothermal with a temperature of 200 °C after that with a sintering process temperature of 900 °C. The polymer used was obtained from whole Stichopus hermanni which was dried by the freeze dried method and milling was carried out using HEM so that it was micro-sized.
Making granules is done by freeze dry method. The first thing to do is to make a 5% HA-TCP solution obtained from 5 g of blood clam shells (Anadara granosa) dissolved in 50 ml distilled water. Furthermore, the manufacture of polymers by dissolving 10 g gelatin and the addition of Stichopus hermanni powder (0.4%; 0.8%; 1.6%) into 50 ml of distilled water. The two solutions are mixed in a ratio of 1:1, stirred and put into a mold. The next step is cooling to -80 °C and drying using the freeze-drying method. The final stage of making this scaffold is sterilization with gamma ray irradiation of 25 kGy by BATAN.14

The research was then conducted following approval from the Ethical Commission for Animal Subjects Faculty of Dental Medicine, Airlangga University, Surabaya No.002 / HRECC.PODM / 1 / 2018. The study began with acclimatization of Wistar rats for 7 days. The division of groups is randomly. Experimental animals were fasted at midnight before being anesthetized using 10% ketamine at a dose of 0.1 cc / kgBB and xylazine 0.01 cc / 100 g BW on the right upper thigh (intramuscular).15 After that cleaning was carried out in the extraction area with water spray and antiseptic fluid for asepsis of the extraction area. Extraction one tooth of the mandibular left in a rat using a needle holder. Application with treatment material was done by dividing 24 male rats into 4 groups. The control group only applied HA-TCP powder from AG shell synthesis, and the AGSH1-3 group applied granules from a combination of Anadara granosa shells and Stichopus hermanni with a concentration of 0.4%; 0.8% and 1.6%. Suturing is done to close the socket using a silk braid (USP. 3/0) from DR. SELLA®. Novalgin® 0.09cc / 200gr BB analgesics and interflex® 0.1cc / 100gr BB antibiotics are needed to control swelling and pain. 14 days after application to the socket, experimental animals were sacrificed and os mandibular preparations were taken and fixed with 10% formalin buffer solution. The next stage is decalcification using ethylenediaminetetraacetic acid (EDTA) for 1 month. The os mandibular specimen is made in the form of a transferal cut preparation by hematoxillin eosin (HE) staining. After that, observations of the number of blood vessels in the socket area with a light microscope (Olympus® CX21, Japan) at 100X magnification. Data tabulation and statistical analysis were then performed with One-way ANOVA followed by the HSD-tukey test.

RESULT

Angiogenesis is always occurs in every healing process, including socket healing. See figure 1. In this study the condition was seen by the presence of blood vessels in the socket preparation. Average of the number of blood vessels in the AG group = 6.50 ± 3.94; AGSH1 = 8.5 ± 2.74; AGSH2 = 13.17 ± 2.56; AGSH3 = 10.33 ± 3.26. Characteristics of blood vessels is the formation of a lumen surrounded by a layer of endothelial cells seen in the os femur region that has defects (Figure).

Figure 2. shows that the lowest was in the AG group, while the highest was in the AGSH2 group.
Observation of the number of blood vessels was evaluated on the 14th day after application to the socket. The ANOVA test found a significant difference in the mean number of blood vessels (p = 0.011). The next statistical test is to compare one group to another through the LSD test.

<table>
<thead>
<tr>
<th>Tabel 1. Hasil uji post-hoc (LSD)</th>
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<tr>
<td>Group (I)</td>
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<td>AGSH2</td>
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</table>

Information: This table shows a comparison of the number of blood vessels between groups. The number of blood vessels in the AGSH group was greater than the AG group. The negative difference means that the area of woven bone is smaller. AGSH2 group has the most number of blood vessels compared to other groups.

Based on the LSD test results, significant differences were only shown in the AG group with the AGSH2-3 group, and AGSH1 group with AGSH2 group.

DISCUSSION

Bone grafting materials are frequently applied in socket preservation and intrabony defect regeneration. The success of bone regeneration in this procedure is determined by the formation and migration of blood vessels from adjacent vital bones into the bone grafting material. Indeed, failure of rapid revascularization will inhibit the process of tissue regeneration. Not only will there be a lack of oxygen and nutrient supply, but cell circulation needed for bone tissue formation will also be hampered.19

Initiation of angiogenesis occurs due to local release of pro and anti-angiogenic growth factors by endothelial cells, in response to injuries that induces Hypoxia-Inducible Factor-1a (HIF-1a) in osteoblast.19,20 This condition expresses vascular endothelial growth factor (VEGF) which has many roles in angiogenesis and osteogenesis.21 The increasing of VEGF activates endothelial cells and promotes vascular permeability. This process (vasculogenesis and angiogenesis) stimulate bone formation by bringing nutrients, oxygen, and minerals needed for mineralization.22 In addition, osteogenic factors, such as Bone morphogenic protein 2 (BMP2), are released from blood vessels, promoting differentiation and mineralization of osteoblasts.21

Besides VEGF, other growth factors that play an important role in the process of angiogenesis are Fibroblast growth factor-2 (FGF-2) and plateletderived growth factor (PDGF), each of which has a different role. VEGF is the initiator of angiogenesis, which is known to mediate the recruitment of endothelial cells, this growth factor is not sufficient for the formation of mature blood vessel tissue. FGF-2 is a mitogen for various types of cells including endothelial cells and mesenchymal stem cells that function to activate endothelial cell proliferation, migration, pericyte traction and matrix production of metalloproteinases, but have little effect on matrix deposition in the process of blood vessel formation. PDGF promotes the maturation of blood vessels by
recruiting smooth muscle cells into the endothelial layer. These three factors show a different sequence during the process of formation of mature blood vessels, where VEGF and FGF-2 stimulate angiogenesis by encouraging recruitment and proliferation of endothelial cells through VEGF and FGF-2, followed by recruitment of mural cells and smooth muscle cells through PDGF. Density of the volume of blood vessels in the alveolar socket shows a significant increase in 7th day and continues to increase at 21st day. In this study, the application of granules in the AG group showed less increasing compared to the group giving the AGSH combination granules with various concentrations, where significant conditions were shown in the AGSH2-3 group. The AG group given granules from HA-TCP synthesized from Anadara granosa shells only provide pores that are connected to each other with gelatinous polymer compounds contained in the womb as an adhesion site for mesenchymal cells to differentiation endothelial cells as the embryo for forming new blood vessels.

Appliation polymeric of HA from Stichopus hermanni can increase the number of blood vessels in the socket. This occurs because of the IIa bond with CD44 and Receptor for Hyaluronan Mediated Motility (RHAMM), a receptor that plays an important role in the regulation of angiogenesis, mainly to influence endothelial cell behavior. The binding of CD44 with HA activates Protein kinase C delta (PKCδ) which leads to RHAMM induction. PKCδ is also required for extracellular-signal-regulated kinase (ERK) activation. This process will induce interactions between RHAMM and ERK. HA also transactivates transforming growth factor beta receptor I (TGFBR1) through PKCδ and ERK. Activation of TGFBR1 by HA is strongly associated with Histone Deacetylase 3 (HDAC3) degradation, which results from tyrosine nitration induced by Rac Family Small GTPase 1 (Rac1). Decreased HDAC3 expression is responsible for the induction of Plasminogen activator inhibitor-1 (PAI-1) and Matrixmetalloproteinase-2 (MMP-2). PAI-1 and MMP-2 activate the process of angiogenesis. Together with VEGF, FGF-2 and PDGF increase the differentiation of endothelial cells to form new blood vessels.

The most effective increase in blood vessels on the 14th day was shown in AGSH2 with a Stichopus hermanni concentration of 0.8%. This is because Stichopus hermanni contains other glycosaminoglycans, such as chondroitin sulphate and keratin sulphate, which can affect the ability of CD44 to bind AH. Modification of bonds due to N and O chains in chondroitin sulfate has a negative effect on the ability of CD44 to bind AH.

The conclusions obtained from this study showed that the combination granules of Anadara granosa shell and Stichopus hermanni could increase the number of blood vessels on the 14th day after tooth extraction. number of blood vessels on the 14th day.

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CERTIFICATE

in appreciation of

Rima Parwati Sari

as Poster Presenter

in

DENTISPHERE 4 - INTERNATIONAL SCIENTIFIC MEETING

5th ASEAN PLUS & TOKUSHIMA JOINT INTERNATIONAL CONFERENCE

"Beyond Dentistry: Synchronizing Science, Technology and Technical Skill in Modern Dentistry"

Chairperson

Dentisphere 4

Vivin Ariesta, drg., Sp.Pro

Lita Agustina, drg., M.H.Kes

Linen