HANG TUAH UNIVERSITY
FACULTY OF DENTISTRY
PRESENT

INTERNATIONAL SCIENTIFIC MEETING

PROCEEDING BOOK

Dentisphere 3

26TH-27TH, AUGUST 2016
SHANGRI-LA HOTEL SURABAYA

Current Concepts and Technology in Improving Dental and Oral Health Care

ISBN 978-602-145-90-1-0

PROCEEDING BOOK INTERNATIONAL SCIENTIFIC MEETING

3rd DENTISPHERE (DENTISTRY UPDATE & SCIENTIFIC ATMOSPHERE) CURRENT CONCEPTS AND TECHNOLOGY IN IMPROVING DENTAL AND ORAL HEALTH CARE

EDITOR :

DRG DIAN WIDYA DAMAIYANTI, M.KES
DRG AGNI FEBRINA P , M.KES
CARISSA ENDIANASARI, S.ST
RIZA FATMA WARDANI, AMD.AK

SETTING/LAY OUT :

DRG. DIAN WIDYA DAMAIYANTI, M.KES
CARISSA ENDIANASARI, S.ST

COVER DESIGN :

MONICA VITA, SKG

PRINTED AND PUBLISHED BY:

FKG HANG TUAH SURABAYA.PRESS
JL. ARIF RAHMAN HAKIM NO.150 SURABAYA 60111
TELP. 031-5945864, FAX. 031-5946261
WEBSITE: www.hangtuah.ac.id
Cetakan : SURABAYA, 2016-06-29

ISBN 978-602-14590-1-0

DEAN OF FACULTY DENTISTRY HANG TUAH UNIVERSITY WELCOME NOTE

Welcome to Surabaya!
Is a great honor for us to welcome you all at the International Seminar "Dentisphere 2016". This international seminar is the third time we have held at the Shangri La Hotel Surabaya. This Seminar which held on 26-27 August 2016 is one of my pride as the Dean of Dentistry Faculty of Hang Tuah University. This is also proofing one of Hang Tuah University's contribution both nationally and internationally in the field of dentistry.

The theme of International Seminar 3rd Dentisphere is "Current Concepts and Technology in Improving Dental and Oral Health Care", which aim is to provide a new generation of dentists who are experts and professionals with the knowledge that continues to grow for the Indonesian nation and the world. We hope that through this event we can raise the professionalism in the field of dentistry for all participants.

I would like to say a very big thanks to our speakers from home and abroad: Japan, Korea, Thailand, and Singapore. Thanks for all contributions and participation and your willingness to come and share your knowledge and experience in dentistry. It is an honor for us that the events will also have an important role in the quality control mechanisms to ensure stability and increased periodically in the field of dentistry.

Also for all the participants, thank you very much for joining the International Seminar 3rd Dentisphere, I hope you can all enjoy the entire summary of the seminar. Hopefully this seminar that we held useful for the advancement of knowledge of dentistry you all peers. I apologize if there are less pleasing for the organization of this seminar.

Enjoy the 3rd international seminar Dentisphere!

P 2.47

RESEARCH ARTICLE

Expression of Osteopontin And Osteoblasts After Given Alloplast With PRF Compare To Xenograf With PRF On Bone Defect

Hansen Kurniawan*, Iwan Ruhadi, Noer Ulfah****
*Department of Periodontics, Faculty of Dentistry, Hangtuah University Surabaya
** Department of Periodontics, Faculty of Dentistry, AirlanggaUniversity Surabaya

ABSTRACT

Background: *Bone grafts have been widely used to repair bone defects, which are caused by trauma, tumor resection, degeneration due to pathological processes, and congenital bone defects. Type of bone graft is classified into several types, autograft, allograft, xenograft, and alloplast. PRP and PRF derived from auto logous blood which sendsa high concentration of growth factors on bone defect area. Osteopontin (OPN) is a phosphorylated acidic glycoprotein in the extracellular matrix of mineralized tissues and is one of the plenty fulnon-collagenous proteins in bone matrix produced by osteoblasts, osteoclasts, and osteocytes. These proteins containing arginine-glycine-aspartate sequence which is the major integrin-binding site and the supporting bone cell adhesi on non mineral matrix .* ***Purpose:*** *The aim of this study is to investigate the effect of xenograft with PRF and alloplast with PRF on osteoblast and osteopontin in bone New Zealand rabbits.* ***Methods:*** *The experiment was held by Post Test Group design. Twenty seven male New Zealand Rabbits were divided into three group. The first group performed the treatment on the right hind limb be treated with Alloplast and PRF treated , The second group performed the treatment on the right hind limb be treated with xenograft and PRF, and The third group was the control group performed the treatment on the right hind limb was given PRF. After treatment the rabbits were sacrificed. Osteoblast and osteopontin of each group was measured by EDTA method and immunohistochemistry method. All data experiment were analyzed by ANOVA and LSD test (p<0,01).* ***Result:*** *The result showed that no significant difference in the expression of osteopontin (OPN) in the xenograft with PRF group compared to alloplast with PRF group (p =0.985>0.05), while the xenograft with PRF group compared with the control group there were significant differences (p =0.001<0.005) and alloplast with PRF group compared with the control group there were significant differences (p =0.000<0.005). While the results of osteoblasts between alloplast with prf compared with xenografts with prf, significant differences as a result of the process of bone formation to see the results of better bone formation.* ***Conclusion:*** *Alloplast with PRF has the same amount of osteopontin in xenograft with PRF so that it can indicate that the initial phase of the process of bone remodeling have the same initial phase, while osteoblasts on alloplast with PRF had higher numbers than xenografts with PRF, so it can be seen that the process of formation bone on alloplast have better results.*

Keywords : *osteopontin, osteoblast, bone, xenograft, alloplast, PRF, immunohistochemistry method*

Correspondene: *Hansen Kurniawan, Department of Periodontics, Faculty of Dentistry, Hang Tuah University, Jl. Arief Rahman Hakim 150 Surabaya, Phone: (031) 5945864 fax: (031) 5945894. Email: drghansenkurniawan@gmail.com*

BACKGROUND

Periodontitis is defined as an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession, or both." The clinical feature that distinguishes periodontitis from gingivitis is the presence of clinically detectable attachment loss. This often is accompanied by periodontal pocket formation and changes in the density and height of subjacent alveolar bone..[1] factors involved in bone destruction in periodontal disease is bacterial plaque. Products of plaque bacteria induce differentiation of progenitor cells into osteoclasts and bone stimulates gingival cells to release mediators which have the same effects .[2] first treatment odf periodontitis is performed to control bacteria plaque and boost the immune system against periodontal bacteria. The treatment fo periodontitis is oral health education , scaling, and root planning.[3]

Periodontal flap provides access for operators to clean granulation tissue and regenerate the periodontal tissues.[3] Bone grafts have been widely used for the repair of bone defects, which are caused by trauma, tumor resection, degeneration due to pathological processes, and congenital bone defects. The use of bone graft to reconstruct bone defects due to periodontitis has been used since 1923 by Hegedus, and reused by Nabers and O'Leary in 1965.[4]

Osteopontin (OPN) is a phosphorylated acid glycoprotein in the extracellular matrix of mineralized tissue and is one of the abundant non-collagenous proteins in bone matrix produced by osteoblasts, osteoclasts, and osteosit.[5]

METHODS

Twenty-seven New Zealand male rabbits aged 4-5 months, weigh 1550 to 2500 grams. All rabbits adjusted during the week. Twenty-seven rabbits were randomly divided into three groups, Group A, B, and C. All groups were sacrificed after 1 month and surgery for bone-making osteoblasts legs for later visits and osteopontin (OPN) under a microscope.

The first group performed the treatment on the right hind limb, treated by Alloplast and PRF. The second group performed the treatment on the right hind limb, treated by Xenograft and PRF. The third group was the control group performed the treatment on the right hind limb treated by PRF. Rabbits is given intramuscularly anaesthetized with ketamine injection. Then the rabbit right tibia fur removed to make it easier to do incision. Before the incision, the rabbits given an injection of one cc lidocaine, local anesthetic in order to strengthen ketamine anesthetic. After that, using rasparotorium for separation of muscle tissue to get tibia bone of rabbits. Bone defects created by using low speed bur with size 3x5 mm and a depth of about 3 mm.

In the first group, the defect is made in the right leg of the rabbits and will be given Alloplast with PRF, while the second group was given Xenograft and PRF in right foot. Then sewing back on the incision and 27

rabbits will be wait for 21 days. After 21 days, we expected at rabbits are already found osteoblast cells and osteopontin cell (OPN).

To sacrifice first anaesthetized animal. Right tibia tissue removed and a cut with a small saw, then put in 70% buffered formalin solution so that the tissue does not rot, tissue hardening, increases the refractive index of the various components tissue and increases the affinity tissue against paint materials. After fixation, the tissue rinsed with water for 6-9 hours and then put in a solution decal. After that, the tissue rinsed with water than put the tissue in a solution of 5% HNO3 decalcified for 60 minutes. Furthermore, process of making preparet follows:

1. Dehydration (water and tissue uptake)
2. Clearing (purification)
3. The impregnation at a temperature of 56 degrees Celsius in paraffin bedding in paraffin
4. Cut using microtome with 4 microns thickness. Then with a DAB staining (deamino benzidine) do observations of the number of osteoblasts and osteopontin expression quantitatively using EDTA staining and immunohistochemical techniques. Data taken from the treatment group and the control group were analyzed using descriptive statistics and one way Annova test.

RESULT

In the research data and analysis of data from research objectives conducted in a number of experimental animals, 27 male new Zealand rabbits aged 4-5 months, weighing 1500 to 2500 grams obtained normal distributed data (Asim sig> 0.05). In data obtained osteopontin note that the data were normally distributed (P = 0981> 0.05) and data on osteoblasts also in normal distribution (P = 0.167> 0.05) .On the results of homogeneity also got results homogeneous distribution data on osteoblasts (P = 0132> 0.05) and in osteopontin (P = 0253> 0.05).

After the statistical analysis of one-way Annova and showed no significant difference expression of osteopontin (OPN) in the Xenograft with PRF than the group Alloplast with PRF (p = 0985> 0.05), whereas in the group Xenograft with PRF compared with the control group there a significant difference (p = 0.001 <0.005) and in the group PRF with alloplast than the control group there were significant differences (p = 0.000 <0.005).

On the results of the analysis on osteoblasts showed the group Xenograft with PRF than the group Alloplast with PRF (p = 0.041 <0.05), there are significant differences, whereas in the group Xenograft with PRF compared with the control group there was no significant difference (p = 0. 996 > 0.005) and in the group with PRF alloplast than the control group there were significant differences (p = 0.034 <0.005).

The rabbits osteoblast and osteopontin normalitas test

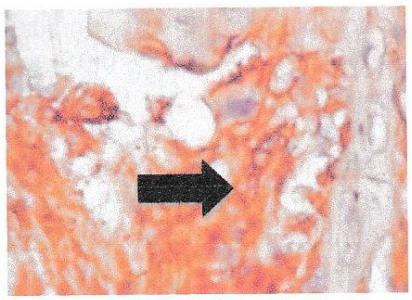
	Normalitas	Homogenitas
Osteopontin	0.981	0.253
Osteoblast	0.167	0.132

The rabbits osteopontin cell Annova test

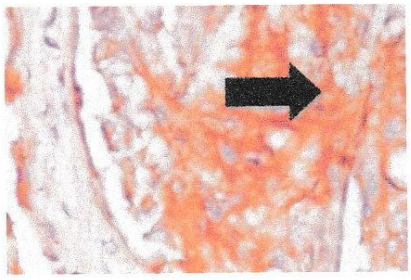
	Alloplast	Xenograft	Kontrol
Alloplast		0.985	0.000
Xenograft	0.985		0.001
Control	0.000	0.001	

The rabbits osteoblast cell Annova test

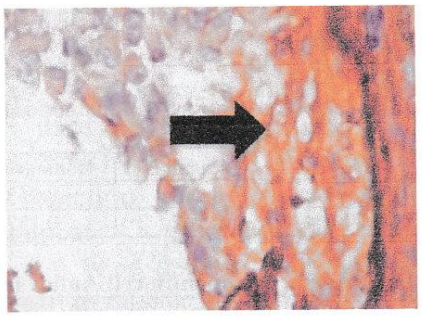
	Alloplast	Xenograft	Kontrol
Alloplast		0.041	0.034
Xenograft	0.041		0.996
Control	0.034	0.996	



Histological examine with immunohistokimia kit staining of osteopontin cell on Xenograft with PRF group



Histological examine with immunohistokimia kit staining of osteopontin cell on Alloplast with PRF group



Histological examine with immunohistokimia kit staining of osteopontin cell on control group

DISCUSSION

In this study aims to determine differences in the number of osteoblasts, and osteopontin (OPN) in bone defects of New Zealand Rabbit with the use of Alloplast with PRF compared xenograft with PRF.

Based on immunohistochemical examination, found the number of osteopontin in the control group (PRF) is lower than the levels of osteopontin from Alloplast with prf group compare to Xenograft with prf group . Whereas in the group Alloplast with prf than Xenograft with prf group did not have significant differences so that it can be seen that the levels of ostepontin at Alloplast with prf and Xenografts with prf is the same level. In this study, it is known that OPN with a combination of hydroxyapatite and calcium oxalate, OPN may help cell attachment to the crystal structure mineral.[6] Expression of OPN by activated macrophages and lymphocytes occurs as part of the host defense mechanism, in which OPN acts as a cytokine which stimulates cellular and humoral of the immune system OPN resulting macrophages and can protect the mineralized tissue on fracture of teeth and bones. In these circumstances, it can be seen OPN aid cell attachment in tissue repair and implant integration .[6] OPN increase in Alloplast with prf and Xenograft with prf showed that the initial phase is well established for bone remodeling, while levels of osteoblasts showed levels of osteoblasts in Alloplast with prf compared with results showing Xenograft with prf. Alloplast with prf to have more osteoblasts that form bone formation better.

This is supported by the statement that in the early stages of osteoblastic cells migrate to the bone surface and begin the secretion of matrix reserbsion and manufacture of the cement layer on the surface of the bone is exposed. The cement layer of collagen fibrils are very few but rich OPN, and sometimes contain bone sialoprotein. Initially, OPN on cement layer can be used for adhesion of osteoblasts, or to start the initial process of calcification. At this stage OPN move to mineral or organic group of bones as starters start a new cycle of mineralization. Studies are often conducted to look at the interaction of macromolecules in an area that allows for calcification, cell attachment and new bone matrix adhesion to the base layer. After the cement layer, which accumulate in the bone matrix lacuna of a secret network becomes fully osteoblast activity. In particular, the cement layer matrix structure in the main areas of bone remodeling observed in alveolar bone of the periodontium. In addition, the main mineral Association of OPN function in connecting matrix and mineral, and regulate the growth of hydroxyapatite crystals in space interfibrillar of bone matrix. The main thing, the physical properties of bone matrix is in large measure determined by the relation of minerals either within or between collagen fibrils. Where OPN, plays a role in determining the biomechanical properties of bone. [6]

Osteopontin (OPN) is a protein multifunctional, and can be seen clearly on the bone, and can also be seen clearly in various cell types including macrophages, endothelial cells, smooth muscle cells and epithelial cells, OPN is involved in two physiological processes and pathological in some organs or networks such as biomineralization, inflammation, leukocyte recrutment and cell survival. OPN is intimately involved in the regulation of both physiological and pathological mineralization. In normal bone tissue, OPN is involved either by osteocast and osteoblasts are the cells responsible for bone remodeling.[6]

Based on the results of the study, osteopontin was no significant difference between Alloplast with prf groups than xenografts with prf groups due osteopontin as an early phase in the process of bone formation as the new bone adhesion. While the results of osteoblasts between Alloplast with prf and Xenografts with prf significantly different as a result of the process of bone formation to see better bone formation. This is consistent with the results of research by sodek, OPN move to mineral or organic group of surface bones as the beginning of a new cycle started mineralisasi.[6]

CONCLUSION

Alloplast with PRF has better results compared with Xenografts with PRF seen from the increase in osteoblasts.

REFERENCES

1. Draidi MA. 2009. *Differences in amount and architecture of alveolar bone loss in chronic and aggressive periodontitis assessed through panoramic radiographs*. Jordan.
2. Newman.MG, Takei HH, Carranza FA, 2011. *Clinical Periodontology 11th edition.* Philadelphia: Elsevier Saunders. P. 63-256; 719-725;785-825
3. Illueca FMA, 2006 .*Periodontal regeneration in clinical practice*. Medical Oral Patology Oral Circular Bucal;11 P.382-92.

4. Sukumar S, 2008. *Bone Grafts in Periodontal.* Therapyacta Medica (Hradec Králové) P.51(4):203–207
5. Muneki Ishijima, Kunikazu Tsuji, Susan R Rittling, Teruhito Yamashita, Hisashi Kurosawa, David T Denhardt, Akira Nifuji, Yoichi Ezura and Masaki Noda,2007 . Osteopontin is required for mechanical stress-dependent signals to bone marrow cells.
6 Sodek, Ganss, and Mckee,. 2000. Osteopontin.Crit rev bio med. Canada. P. 279-303