REVIEW ARTICLE

Demineralized Dentin Material Sponge (DDMS) Promotes RUNX2 expression in Osteoblastogenesis

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ABSTRACT

In cases of severe bone loss, natural healing does not occur because the body's natural regenerative capacity is exceeded. Therefore, there is a need for alternative materials that can support the healing process with good osteointegrative properties. Osteointegration is the relationship between a synthetic biomaterial implanted in the body and the biological response of the host tissue. Osteoblasts play a role in the process of bone remodeling and cooperate with osteoclasts to balance bone formation and resorption and remove mature bone tissue to maintain bone homeostasis. RUNX2 is a member of a small domain of transcription factors that are important for differentiation and proliferation during the transition of mesenchymal stem cells to osteoprogenitor cells during osteoblast differentiation. The demineralized dentin material sponge derived from bovine dentin contains 70% hydroxyapatite, 20% organic matrix, and 10% water, contains organic and inorganic components similar to dentin and human bone, which enhances the expression of RUNX2. It can be used as a bone substitute material. In the process of osteoblast formation. The aim of this study was to determine the potential ability of demineralized dentin sponges to promote RUNX2 expression in osteoblastogenesis.

Keywords: Osteoblast, Osteoblastogenesis, RUNX2, Demineralized Dentine Material Sponge

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INTRODUCTION

The craniofacial area often suffers from complex injuries either due to accidental (traumatic and acute conditions), congenital (birth defects/deformities) and pathological consequences (such as maxillary tumors, ameloblastoma and other infections) that require surgical treatment. In cases with widespread bone loss, self-healing usually cannot occur because it exceeds the body's natural regenerative capacity so that alternative materials are needed that can assist the healing process which have good osteointegration properties, one of which is the porosity property which allows fast absorption of materials in the body and can stimulate the growth of new bone tissue. The addition of growth factors and a few other osteoinductive variables can increment osteointegration (1,2). Osteointegration is the relationship between a syntheticbiomaterials implanted in the body and biological response of the host tissue. The concept of bio-integration can be easily understood through the example of bone grafting using a ceramic scaffold, where the scaffold has a squeeze to integrate bone as well as has a role in the bone remodelling process (3).

The process of bone remodeling stems from the critical role played by osteoblasts and osteoclasts, which work together to balance bone formation and resorption and maintain bone homeostasis by removing mature bone tissue (4). RUNX2 is a member of a small domain of transcription factors that are important differentiation and proliferation during the transition of mesenchymal stem cells to osteoprogenitor cells during osteoblasts differentiation. Transcription factors are proteins that bind to specific DNA sequences in the target gene and then affect the transcription of the target gene positively or negatively. ECM protein binding to integrins, mechanical load, FGF-2, parathyroid hormone (PTH), and bone morphogenetic protein (BMP) regulates RUNX2 expression (5). The use of Collagen Sponge can be used as a hemostatic agent that functions in stopping bleeding and the wound healing process, increasing fibroblast growth and vascularization can increase granulation tissue and cell adhesion so that good healing can occur (6). Collagen can be obtained from several extractions of natural ingredients, one of which

is bovine bone.
The purpose of this study was to determine the potential ability to use demineralized dentin material sponge (DDMS) to promote RUNX2 expression in osteoblastogenesis during the bone healing process.

REVIEW

Osteogenesis

The process of bone formation (osteogenesis) occurs through two main pathways, namely the occurrence of endochondral ossification/intramembranous ossification and specific condensation by mesenchyme stem cells (MSCs). During endochondral ossification, MSCs differentiate into chondrocytes, preserve cartilage tissue and are mineralized by osteoblasts. Chondrocytes and cartilage matrix reach a terminal and hypertrophic state where they are reabsorbed by preosteoblasts, osteoblasts, blood vessels, and are subsequently replaced by bone marrow. Therefore, the process of endochondral ossification is restricted to the formation of bone marrow-containing types in the body, whereas the intermediate chondrogenic stage is induced because intramembranous ossification is osteogenic and leads directly to differentiation of MSC condensates. Instead, this process forms the facial bones and skull (7).

Osseointegration

The use of autograft in the treatment of bone defects has limitations such as number and donor availability. This has triggered efforts to search for alternative bone substitutes with properties and structures similar to real bone. For now, several grafts are commercially available and some of them are still in the testing stage for the osseointegration between the material and the tissue. Currently, many new materials are being developed, materials with porosity tend to increase the capacity of osseointegration compared to solid materials which tend to form a fibrous layers on the surface of the deformity (2).

Osteointegration is a direct relationship between a biomaterial and bone tissue without any soft tissue interposition and can be observed at the histological, molecular and cellular stages. One example of the phenomenon of osteointegration can be observed in the improvement of the condition of the bone given an implant. Cells associated with the surface of the material through the protein layer, resulting in migration and cell adhesion. When a bone defect occurs and implants are placed, platelets are secreted on the implant surface between the fibrin fibres and several growth factors such as platelets, insulin growth factors (IGF-1, IGF-2), fibroblast growth factors (FGF-α, FGF-β), bone morphogenic protein (BMP), serotonin and histamine which promote mesenchymal cell migration, proliferation and differentiation so that they can bind well to the implant surface that has been formed. Several factors can interfere with the osteointegration process including several pharmacological drugs, such as cyclosporine, methotrexate, cisplatin, warfarin and low molecular weight heparin, nonsteroidal anti-inflammatory drugs (NSAIDs) which function to inhibit COX-2 inflammatory mediators (8).

Preosteoblasts and Osteoblasts

Osteoclasts and osteoblasts are two major cell types that regulate the bone remodeling process. Osteoblasts and osteoclasts have opposite properties, osteoblasts play a role in the process of new bone formation and osteoclasts play a role in the process of bone destruction. Under physiological conditions, osteoblasts secrete macrophage colony-stimulating factor (M-CSF). It signals macrophage colony-stimulating factor (Csf1r) through its receptor tyrosine kinase receptor. This factor is expressed in almost all mononuclear phagocytes. Osteoblasts control the availability of M-CSF and RANKL to osteoclast precursors and also regulate the production and secretion of osteoprotegerin (OPG). OPG itself is her receptor for RANKL and a major component of osteoclastogenesis (4).

The bone skeleton is formed by intramembranous and endochondral ossification. During intramembranous bone development, mesenchymal cells differentiate into osteoblasts and bone is directly formed from osteoblasts. In endochondral ossification, cartilage

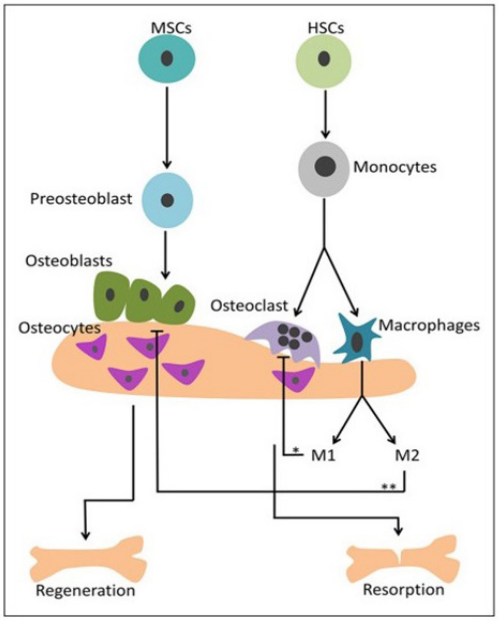

Fig. 1: Osteoblast differentiation process (Peric Kacarevic, et al. 2019)

skeleton is formed by chondrocytes, vascular invasion occurs, and osteoclasts and mesenchymal cells invade cartilage. Differentiated chondrocytes eventually undergo apoptosis, mesenchymal cells differentiate into osteoblasts, and bone forms at the base of cartilage structures, which are then completely replaced by mature bone (8).

Several cytokine and hormone signaling cascades regulate osteoblastogenesis, leading to subsequent activation of downstream transcription factors. Among these downstream transcription factors, RUNX2 is one of the most important transcription factors expressing osteoblastic markers such as alkaline phosphatase (ALP), osteocalcin (OCN), osteopotin (OPN), osteonectin (ONN), bone sialoprotein (BSP) and collagen type 1 alpha 1 chain (COL1A1) in osteoblastogenesis. During osteoblastogenesis, RUNX2 is slightly overexpressed in independent MSCs and increases during preosteoblast proliferation. RUNX2 levels peak during the immature osteoblast stage and decrease during the mature stage. RUNX2 increases the expression of Osteix (OSX/Sp7), a transcription factor important for osteoblast binding and differentiation. Consequently, the transcription factors RUNX2 and OSX are critical for osteoblastogenesis (9).

RUNX2

RUNX2, which belongs to the RUNX protein family consisting of RUNX1, RUNX2, and RUNX3, is a key transcription factor in skeletal development. The RUNX family of proteins have a small domain that directly binds to DNA. RUNX2 is known to heterodimerize with Cbfb and enhance its DNA binding capacity (10). RUNX2 is involved in the proliferation of osteoblast progenitors expressed in mesenchymal cells, RUNX2 expression is regulated at the preosteoblastic stage, and then RUNX2 expression reaches maximum levels during the immature stage of osteoblasts, the final regulation at maturation to form the osteoblastic matrix. It later develops into a bone matrix protein gene (BMP) (11).

Regulation of osteoblastic differentiation by RUNX2 can be divided into two phases: early and mature. In the early stages, RUNX2 type 1 plays a role in supporting the differentiation of adherent mesenchymal cells to preosteoblasts and immature osteoblasts. While RUNX2 will play a role in the maturation phase, namely when the process from immature osteoblasts to mature osteoblasts to become osteocytes. RUNX2 regulation in this maturation phase will be decreased compared to the initial phase because RUNX2 overexpression can inhibit bone growth and lead to osteopenia (11).
RUNX2 has been associated with osteoblast maturation and the formation of mature bone that has resistance to bone resorption, in previous studies an increase in the number of osteoblast differentiation and pre-osteoblast phase can invade blood vessels and cause osteoblast patterns that can be observed from the trabecular pattern in rat cartilage (12).

Bone Graft Material

The use of grafts as bone substitutes can be used to maintain bone healing with a 100% success rate Autogenous bone graft is one of the grafts that can be used as a bone substitute with good results but has the disadvantage of frequent pain and an increased risk of infection in the graft area and cannot be used in large bone defects. Allogeneic grafts has clinical advantages as it does not require a second surgery but may induce a weak immune response. Xenograft is one of the grafts that are often used in the repair of alveolar bone and tooth sockets, maxillary sinus floor elevation and periodontal defects with high predictability and one of the xenografts that is often used is bovine-xenograft (13). One type of bone graft that is often used in dental materials is hydroxyapatite (HA), which is the main component of bone (55-56%) and teeth containing growth factors and is associated with the processes of osteogenesis and osteointegration. The content of HA is also found in dentin, one of which is bovine (cow) dentin, even human bone, human dentin and bovine dentin have the same content which consists of 70% hydroxyapatite, 20% organic matrix and 10% water (14).

Demineralized Dentin Material Sponge (DDMS)

Dentin is the largest part of the tooth and is composed of approximately 70% mineral, 20% organic and 10% liquid. Type I collagen fibers constitute 90% of the organic matrix, and under physiological conditions, dentin collagen fibers are surrounded by hydroxyapatite (15,16).

Demineralized Dentin Matrix (DDM) is a synthetic bone substitute membrane derived from bovine dentin and has a composition similar to human bone. This is the type I collagen complex (COL-I), which can release growth factors such as BMPs and has important osteoinductive and osteoconductive biological effects.

DDM is highly osteoinductive and can induce bone growth simultaneously (17,18). DDM has dentinal tubules with a diameter in the range of 1.2-2.5 mm so that due to its micro size it can be used to induce osteoinductive and osteoconductive processes in bone. DDM is also biocompatible and has no adverse effects on the body, so it can be used as a bone graft material by releasing BMP (14). DDM powder has a particle size of 300-800 m with a dentin tubular pore diameter of 1-3 m. Block-shaped DDM has pores with a diameter of 300-400 m. Due to the microroughness of DDM powder, it was found that the content of rhBMP-2 can be used as a BMP carrier as a BMP carrier. DDM powder itself consists of several organic and inorganic components, so it can be used as a scaffold (19,20).

Based on a previous study by Mulyawan et al. FTIR and SEM projections were performed on DDMS and bovine pericardium collagen membrane (BPCM) and found that DDMS and BPCM share almost the same functional groups and have similarities with human bone. The SEM-DEX test projections showed that the two samples had an irregularly shaped porous structure uniformly distributed over the entire surface resulted from freeze-drying methods. DDM contains (57.923%) carbon,

(32.705%) oxygen, (2.158%) sodium, (0.309%) sulfur, (2.959%) chlorine, (0.325%) calcium and (3.621%) zirconium while BPCM does not contain that content so that DDM can be used in bone regeneration and can even induce bone better than calcified dentin in the implantation process followed by 4 weeks observation (17). Another study found that demineralized dentin material membrane (DDMM) implantation prevented fibroblast proliferation in bone defects characterized by reduced numbers of fibroblasts in the bone defects and possibly promoted bone regeneration. I'm here. These results indicate that DDMM implantation into critical-sized bone defects is a promising therapeutic option for osteogenesis (21).

DDMM can also be used as a bone graft material similar to autogenous bone, it is also can be best choice for membrane material because it is not hard for sampling, halal material, and the its capability to help bone regeneration process (22).

CONCLUSION

The use of DDMS has the potential to promote RUNX2 expression in Osteoblastogenesis and further research is needed in vitro and in vivo to obtain histopathological features and evaluate the gradual increase in RUNX2 expression in osteoblastogenesis.

CONFLICT OF INTEREST

The author declares no conflict of interest regarding the publication of this paper.

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REFERENCES

1. Novais, A.; Chatzopoulou, E.; Chaussain, C.; Gorin, C. The Potential of FGF-2 in Craniofacial Bone Tissue Engineering: A Review. Cells.2021;10(4): 932. doi : 10.3390/cells10040932
2. Paulo MJ, dos Santos MA, Cimatti B, Gava NF, Riberto M, Engel EE. Osteointegration of porous absorbable bone substitutes: A systematic review of the literature. Clinics (Sao Paulo). 2017 Jul; 72(7): 449–453. doi : 10.6061/clinics/2017(07)10
3. Fernandez, F. B., Babu, S. S., Komath, M., & Varma, H. Biointegration of bone graft substiutes from osteointegration to osteotranduction. Biointegration of Medical Implant Material. 2020;245–261. doi:10.1016/b978-0-08-102680-9.00010-x
4. Adamopoulos, I. E. Inflammation in bone physiology and pathology. Current Opinion in Rheumatology. 2018;30(1), 59–64. doi:10.1097/bor.0000000000000449
5. Sato Hirotaka, Takaoka Yutaka. RUNX2 expression during early of tooth-extraction wounds in rats. Journal of Oral Science. 2015;57(4):319-325 doi:10.2334/josnusd.57.319
6. Bakır, H., Uysal, E., Kurt, A. H., & Kirdak, T. (2016). Analysis of the Effect of Locally Applied Bovine Collagen Sponge and Adipose-Derived Mesenchymal Stem Cells on Seroma Development in Rats Undergone Mastectomy and Axillary Dissection. Journal of Investigative Surgery.2016;30(4):252–259. doi:10.1080/08941939.2016.1236856
7. Perić Kačarević Ž, Rider P, Alkildani S, Retnasingh S, Pejakić M, Schnettler R, Gosau M, Smeets R, Jung O, Barbeck M. An introduction to bone tissue engineering. Int J Artif Organs. 2020 Feb;43(2):69-86. doi: 10.1177/0391398819876286
8. Stanislav Bondarenko, Ahmed Amine Badnaoui, Yuriy Prudnikov. Osteointegration of Implants and Risk Factors of Disorders(Literature review). Anatomy Physiol Biochem Int. 2018; 4(3): 555638. doi: 10.19080/APBIJ.2018.04.555638
9. Amarasekara, D. S., Kim, S., & Rho, J. Regulation of Osteoblast Differentiation by Cytokine Networks. International Journal of Molecular Sciences.2021;22(6): 2851. doi:10.3390/ijms22062851
10. Komori T. Roles of Runx2 in Skeletal Development and Cancer. Adv Exp Med Biol. 2017;962:83-93. doi: 10.1007/978-981-10-3233-2_6
11. Komori T. Regulation of Proliferation, Differentiation and Functions of Osteoblasts by Runx2. Int J Mol Sci. 2019 Apr 4;20(7):1694. doi: 10.3390/ijms20071694
12. Kim, J.-M., Yang, Y.-S., Park, K. H., Ge, X., Xu, R., Li, N., … Shim, J.-H. (2020). A RUNX2 stabilization pathway mediates physiologic and pathologic bone formation. Nature Communications. 2020. 11(1). doi:10.1038/s41467-020-16038-6
13. Merli, M., Mariotti, G., Pagliaro, U., Mazzoni, A., Moscatelli, M., & Nieri, M. The Fence Technique: 100% Autogenous Bone Graft vs 50% Deproteinized Bovine Bone Matrix and 50% Autogenous Bone Graft. A Histologic Randomized Controlled Trial. The International Journal of Periodontics & Restorative Dentistry. 2020;40(2):181–190. doi:10.11607/prd.4116
14. Soesilawati, P.; Yuliati, Y.; Nastiti, A.P.; Abu Kasim, N.H. Demineralized Dentine Material Membrane As Barrier Membrane for Bone Regeneration. Preprints. 2021:202103044. doi: 10.20944/preprints202103.0441.v1
15. Islam, Md Sofiqul & Aryal Ac, Smriti & Nassar, Mohannad & Tagami, Junji. Bio-Modification of Demineralized Dentin Collagen by Proanthocyanidins-enriched Cranberry: An In-Vitro Study. J Int Dent Med Res 2021; 14(3): 919-924). Available from: Internet address

16. Takahashi N, Nyvad B. Ecological Hypothesis of Dentin and Root Caries. Caries Res. 2016;50(4): 422-431. doi:10.1159/000447309
17. Mulyawan, Indra & Danudiningrat, Coen & Soesilawati, Pratiwi & Aulani, Aulanni'am & Yuliati, Anita & Suroto, Heri & Bramantoro, Taufan & Rizqiawan, Andra & Moon, Seong-Yong. (2022). The Characteristics of Demineralized Dentin Material Sponge as Guided Bone Regeneration Based on the FTIR and SEM-EDX Tests. European Journal of Dentistry. 2022 Mar 13. doi: 10.1055/s-0042-1743147
18. Mulyawan I, Danudiningrat CP, Soesilawati P, Aulanniam , Yuliati A, Suroto H et al. Demineralized Dentin Material Sponge as a Guided Bone Regeneration. Malaysian Journal of Medicine and Health Sciences. 2021 Sep;17:98-101. Available from: Internet address
19. Um, In.-Woong., Kim, Young.-Kyun. and Mitsugi, M. Demineralized dentin matrix scaffolds for alveolar bone engineering. The Journal of Indian Prosthodontic Society. 2017;17(2): 120–127. doi: 10.4103/jips.jips_62_17
20. Um IW, Ku JK, Kim YK, Lee BK, Leem DH. Histological Review of Demineralized Dentin Matrix as a Carrier of rhBMP-2. Tissue Eng Part B Rev. 2020 Jun;26(3):284-293. doi: 10.1089/ten.TEB.2019.0291.
21. Soesilawati, P.; Nastiti, A.P.; Firdauzy, M.A.B.; Yuliati, Y.; Kasim, N.H.A. (2021). Regulating The Fibroblast Growth Into Critical Size Mandibular Bone Defect via Demineralized Dentin Material Membrane Implantation. Internasional Journal of Pharmacetucal Research. 2021;13 (1): 3758-3763. Available from: Internet address
22. Soesilawati, Pratiwi.; Tantiana.; Zahra, Annisa (2021). Anti Immunogenicity Evaluation of Bovine Demineralized Dentine Membrane Material. Malaysian Journal of Medicine and Health Science. Mal J Med Health Sci 17(SUPP2). 2021 April: 103-105. Available from: Internet address

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