Trop J Nat Prod Res, August 2025; 9(8): 3635 - 3639 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Polymethylmethacrylate-Hydroxyapatite (PMMA-HA) Increased Osteogenesis via a Stimulation of BMP-2 and FGF-2 Expression in Experimental Rats (*Rattus norvegicus*)

Lambang Bargowo[1,2], Chiquita Prahasanti[1]*, Clarinta P Widjojo[3], Jonathan Setiawan[3], Banun Kusumawardani[4], Mohammed A Aljunaid[5,6], Huda R Qaid[2,6], Mohammed S Al-Ak'hali[7]

[1]*Department of Periodontology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia*
[2]*Doctoral Program, Faculty of Dental Medicine, Airlangga University, Surabaya, Indonesia*
[3]*Student of Periodontic Residency Program, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia*
[4]*Department of Biomedical Sciences, Faculty of Dentistry, University of Jember, Surabaya, Indonesia*
[5]*Department of Oral and Dental Medicine, Faculty of Medicine, Taiz University, Taiz, Yemen*
[6]*Faculty of Oral and Dental Medicine, Al-Saeed University, Taiz, Yemen*
[7]*Department of Preventive Dental Sciences, College of Dentistry, Jazan University, Saudi Arabia*

ARTICLE INFO

Article history:
Received 08 March 2025
Revised 12 June 2025
Accepted 11 July 2025
Published online 01 September 2025

ABSTRACT

Polymethylmethacrylate (PMMA) has been used for a long time as an implant material in orthopaedic surgery. PMMA lacks the bioactivity necessary for effective osseointegration. Unlike PMMA, hydroxyapatite (HA) offers better osteoconductive, bioactive, and biocompatible properties. HA can be derived from limestone ($CaCO_3$) through processing at Balai Besar Keramik (BBK) or from bovine bone following the Good Manufacturing Practice (GMP). This study aimed to evaluate the osteogenic potential of PMMA-HA implants by analyzing bone morphogenetic protein 2 (BMP-2) and fibroblast growth factor 2 (FGF-2) expression in a rat bone model. The study consisted of six groups: a control group for 7 days, a control group for 14 days, PMMA-HA (BBK) group for 7 days, PMMA-HA (BBK) group for 14 days, PMMA-HA (GMP) group for 7 days, and PMMA-HA (GMP) group for 14 days were implanted into the femurs of the rats at a ratio of 83.8:16.2. Immunohistochemical assays were conducted on days 7 and 14 to assesses FGF-2 and BMP-2expression levels. The expression of FGF-2 and BMP-2 was elevated at 7 and 14 days after implanting PMMA-HA (BBK) and PMMA-HA (GMP) into the femur of Wistar Rats. The application of PMMA-HA (BBK) and PMMA-HA (GMP) into the femur of Wistar Rat resulted in an upregulation of FGF-2 and BMP-2 expression, suggesting that this biomaterial has the potential to enhance bone regeneration by promoting osteogenesis.

Keywords: BMP-2, FGF-2, Polymethylmethacrylate, Hydroxyapatite, Osteogenesis

Introduction

Polymethylmethacrylate (PMMA) has been used for a long time as an implant material in traumatology and orthopaedic surgery. However, the lack of bioactivity is one of the shortcomings that has limited the use of PMMA.[1] Hydroxyapatite (HA) is a bioceramic material that closely resembles the structure of bone apatite. It can be synthesized from natural sources such as limestone or calcium carbonate ($CaCO_3$). HA is highly biocompatible, osteoconductive, and osteoinductive, and promotes osseointegration. Consequently, it is widely used as a regenerative material in dental implants.[2] Several studies have proven that adding HA material to the PMMA matrix increases the osteoblast response compared to using PMMA alone.[3–5] PMMA-HA composite increases shear strength at the implant-bone interface after six weeks of implantation. [6,7]

*Corresponding author. Email: chiquita-p-s@fkg.unair.ac.id
Tel: +62 31 5030255

Citation: Bargowo L, Prahasanti C, Widjojo CP, Setiawan J, Kusumawardani B, Aljunaid MA, Qaid HR, Al-Ak'hali MS. Polymethylmethacrylate-Hydroxyapatite (PMMA-HA) Increased Osteogenesis via a Stimulation of BMP-2 and FGF-2 Expression in Experimental Rats (*Rattus norvegicus*). Trop J Nat Prod Res. 2025; 9(8) 3635 – 3639 https://doi.org/10.26538/tjnpr/v9i8.24

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Osteoblasts are essential for bone production as they secrete chemokines, prostaglandins, and many growth factors, including bone morphogenetic protein (BMP), Transforming growth factor-β (TGF-β), and fibroblast growth factor (FGF). The osteoblasts release these substances and act on nearby cells (autocrine) and neighboring cells (paracrine), regulating the activity of osteogenic and osteoclastic cells.[8] BMP-2 can stimulate the transformation of mesenchymal stem cells into osteoblasts. BMP-2 is a part of the TGF-β superfamily and is involved in various biological processes such as embryogenesis, development, and bone regeneration.[9] Furthermore, FGF is crucial in tissue repair, regeneration, and osteogenesis. FGF-2 induces angiogenic activity and proliferation of undifferentiated mesenchymal cells. In 2001, FGF-2 was first used as a medicine for decubitus ulcers in Japan.[10] Although PMMA-HA implants have beneficial properties, more research is needed to understand their biological functions. To date, no *in vivo* study has been done on combined PMMA-HA implants. Therefore, this study aimed to investigate the osteogenic effect of PMMA-HA implants by examining BMP-2 and FGF-2 expression.

Materials and Methods

Animals

Male Wistar rats were obtained from the Biochemical Experimental Animal Laboratory, Medical School, Universitas Airlangga. The experimental animals were healthy 16-week-old male Wistar rats with an average body weight of 300 g. The rats were placed in separate cages for 1 week to acclimatize to the laboratory environment. The rats were fed with standard rodent pellets and allowed free access to drinking

water. The handling of the animals complied with the National Animal Care Guidelines.[11]

Ethical approval
Ethical approval was granted by the Faculty of Dental Medicine, Universitas Airlangga, under reference number 942/HRECC.FODM/XII/2022.

Study design
This study employed a laboratory experimental design with a post-test-only control group. The animals were divided into six groups, each consisting of 7 rats. The groups include control groups (C7 and C14) and treatment groups (BBK7, BBK14, GMP7, and GMP14). Observations were made on day 7 and day 14 post treatment. Distinct markings were applied to each rat for group identification.

Preparation and sterilization of PMMA-HA implant material
PMMA was sourced from Cemex® System Genta (Tecres- Italy) and mixed with hydroxyapatite (HA) derived from two sources: from limestone processed by Balai Besar Keramik Indonesia (HA-BBK) and from bovine bone processed under Good Manufacturing Practice (GMP) at the Tissue Bank of Dr. Soetomo, Indonesia (HA-GMP). The mixing ratio of PMMA to HA was 83.8:16.2. To prepare the mixture, 0.1 g of PMMA-HA powder was combined with 0.016 mL of monomer and stirred with a cement spatula on a dappen glass until it reaches a dough-like consistency over 1 - 1.5 minutes. Then the mixture was molded into nylon molds measuring 2 mm in height and 1 mm in diameter and left to set for 5 - 10 minutes. Subsequently, the samples were subjected to three washes with phosphate-buffered saline (PBS) followed by immersion in 70% ethanol for 2 h and exposure to UV for an additional 2 h.[3]

PMMA-HA implantation procedure in Wistar rats
Prior to the procedure, the rats were anesthetized using a combination of 10% ketamine and 0.2 mL of xylazine (0.2 mL per 200 g body weight), administered intramuscularly. Following anaesthesia, the femur area was shaved and cleaned with 70% alcohol. A 10 mm incision was then made using a #15C blade on the lateral aspect of the femur, extending down to the bone surface. Drilling was performed with saline irrigation at a speed not exceeding 1500 rpm, adhering to the length and diameter specifications of the implants. The implants were subsequently placed into the osteotomy site on the lateral femur surface until primary stability was achieved. In the control group, which did not receive implants, drilling was conducted to the same depth and diameter, followed by wound closure using simple interrupted sutures. Evaluation of implant integration and biomarker expression was conducted on day 7 and day 14 post-implantation. At the end of these observation periods, the animals were euthanized under deep anaesthesia using 10% ether. The femurs from both control and treatment groups were then harvested and dissected for subsequent immunohistochemical analysis.

Immunohistochemistry
After euthanizing the rats, immunohistochemical analysis was performed to detect BMP-2 and FGF-2 expression in the implanted PMMA-HA material. Monoclonal antibodies targeting BMP-2 (sc-74412 Santacruz Biotech US) and FGF-2 (sc-137087 Santacruz Biotech US) were applied at dilutions of 1:100 and 1:100, respectively, as per the manufacturer's recommendations and standard IHC protocols.[12–14] The samples were observed under an Olympus LX-31 light microscope equipped with a Lumic 6X-8 digital camera at magnifications of 100x, 400x, and 1000x. Positive expression was indicated by brown staining, representing antigen-antibody binding on immunoreactive cells, which was then quantified for analysis.

Statistical analyses
Statistical analyses were conducted using SPSS version 25. Variations in BMP-2 and FGF-2 expression were assessed using One-way ANOVA test followed by Tukey's HSD post hoc test. A p-value less than 0.05 was considered statistically significant for each group.

Results and Discussion

The BMP-2 expression results are illustrated in Figures 1 and 2. The findings demonstrate an increase in BMP-2 levels from day 7 to day 14 across all groups, reflecting progressive osteogenic activity over time. PMMA-HA (BBK) and PMMA-HA (GMP) groups exhibited higher BMP-2 expression levels compared to the control group at both time points, with the PMMA-HA (GMP) group showing the highest mean BMP-2 level on day 14. These findings suggest that both PMMA-HA implant materials may promote osteogenic differentiation, with PMMA-HA (GMP) demonstrating a slightly higher osteoinductive effect.
There was a significant difference in the mean FGF-2 expression on the seventh day between the control group, and the BBK and GMP groups. The average FGF-2 expression level in the BBK group was more than that of the GMP group on the seventh day, whereas the average FGF-2 expression level in the GMP group was greater than that of the BBK group on the fourteenth day. Nevertheless, no statistically significant disparity was observed between the two groups.
ANOVA test results were obtained on the 14th day, depicted in Figure 2, indicated that the GMP group had higher BMP-2 and FGF-2 expression levels. A notable and significant disparity in the average FGF-2 expression was observed between the control group and GMP, and between the control group and BBK. However, no significant variation in the mean FGF-2 expression was observed between the BBK and GMP groups.
The GMP group had the highest value based on the average expression of the two markers on the 14th day. The GMP group showed a notable increase in the expression levels of both markers on the 7th and 14th day compared to the control group.
Additionally, there was a significant difference in the expression level of the BMP-2 marker between the BBK and GMP treatment groups on the seventh and fourteenth day, with the GMP group showing the highest expression level. No significant differences were observed in the expression of FGF-2 marker between the two treatment groups (BBK and GMP) on the seventh and fourteenth day.
Both treatment groups had significantly higher expression levels of BMP-2 and FGF-2 than the control group. These results indicate that both PMMA-HA (BBK) and PMMA-HA (GMP) can increase the production of BMP-2 and FGF-2 markrs in osteoblast cells when exposed to these chemicals.
PMMA is a stiff polymer with favourable biocompatibility and mechanical characteristics, although it has limited ability to connect with bone. HA is a gold-standard material widely used as a biomaterial mixture because of its non-toxic, non-inflammatory, biocompatibility, and bioactive properties.[15] HA is a permeable bioceramic that facilitates the proliferation of capillaries and blood vessels. The large supporting pores of the HA scaffold stimulate osteogenesis due to better vascularization and high oxygen permeability.[16]
The contact between the bone and the implant surface, known as osseointegration, is an important factor in the success of implant placement. Osseointegration refers to the fusion of the bone with the implant surface where there is no movement between the implant and the bone. Biocompatibility of the implant surface is an important factor in the integration process.[17] The use of HA in implants shows greater osseointegration between bone and implant compared to implants without HA. HA, which is conductive, causes osteogenic cell morphogenicity to become conducive and accelerates bone formation and maturation.[18]
This PMMA-HA material will stimulate osteoblast cells to release BMP-2, which converts mesenchymal stem cells (MSCs) into mature osteoblasts. Osteoblast releases BMP-2 and binds to BMP receptors to transmit signals through the Smad pathway. Smad 1/5/8 working together with active Smad4. Activation of Smad regulates the function of the transcription factor RUNX2, leading to the activation of OSX, which subsequently stimulates the increase in osteoblast-specific proteins such as BMP-2 and FGF-2. This expression will enhance the reproduction and specialization of osteoblasts, leading to improved bone formation and facilitates the process of bone integration.[19]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

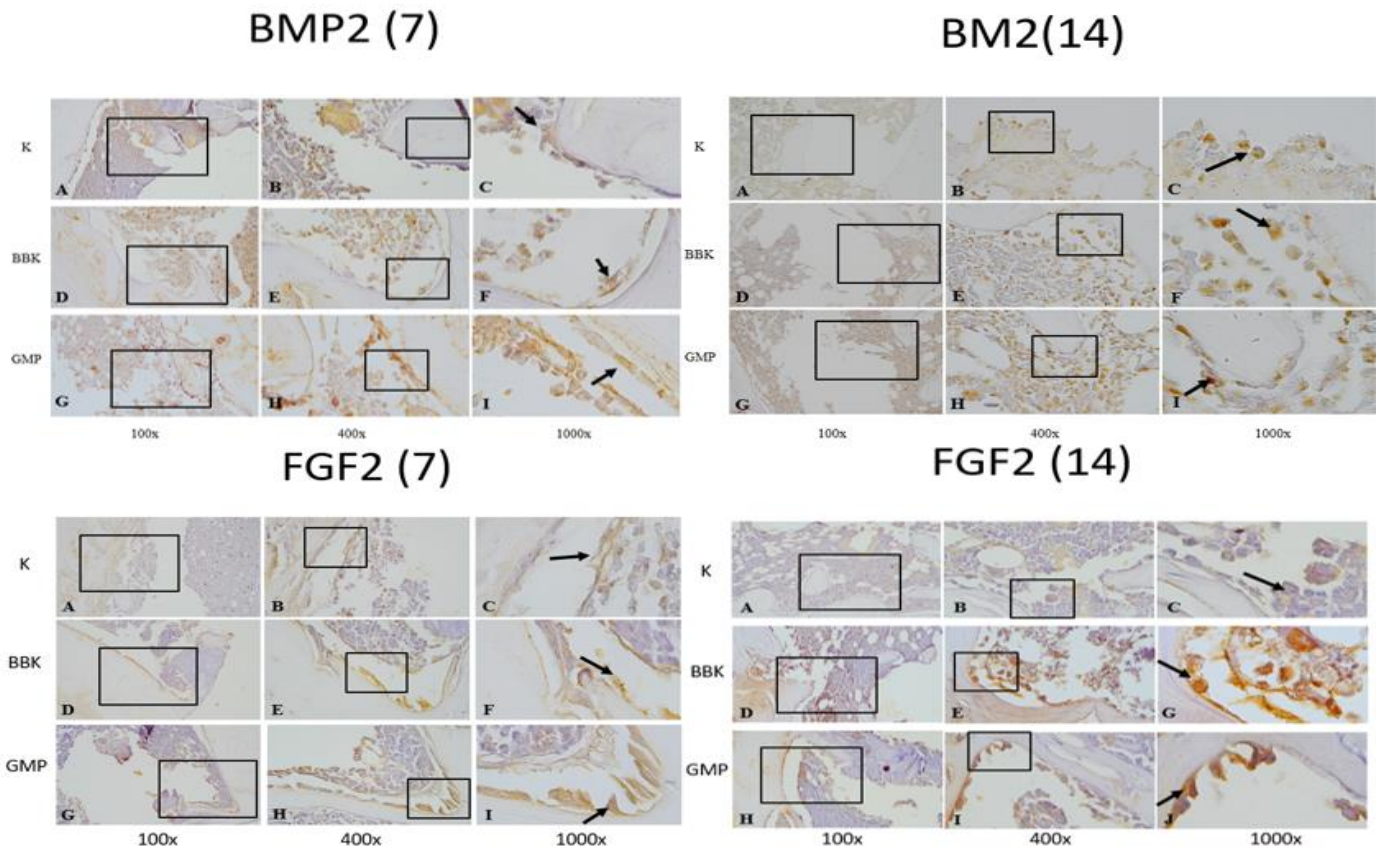

Figure 1: BMP-2 and FGF-2 expression on 7th and 14th day: Control [a-c], PMMA-HA (BBK) [d-f], and PMMA-HA (GMP) [g-i]

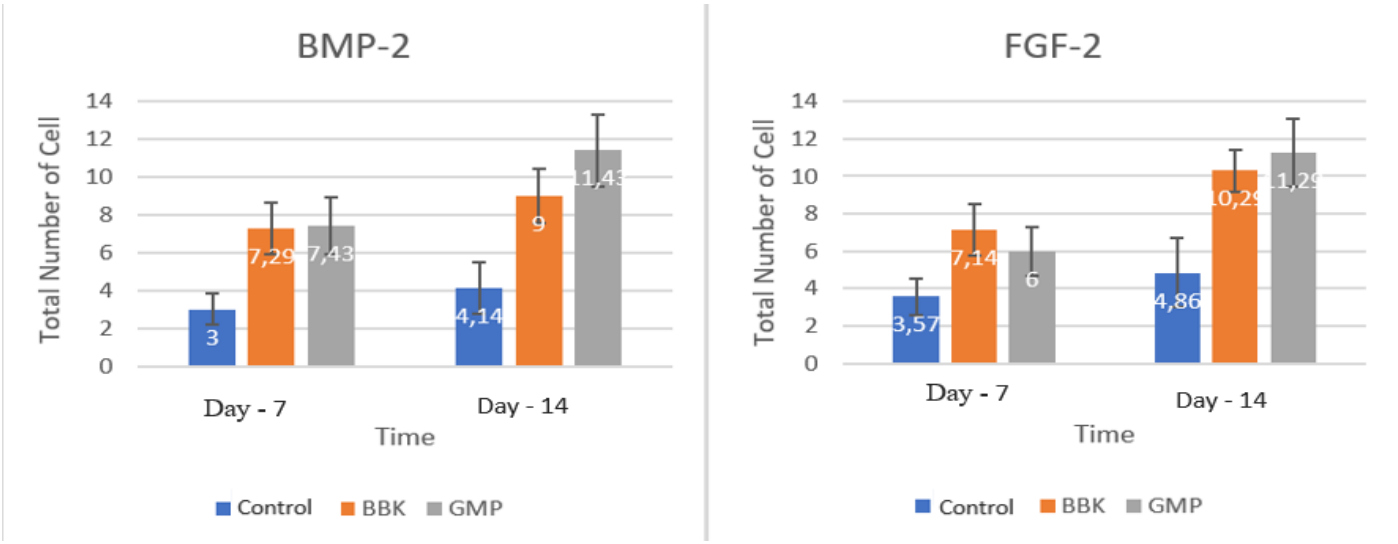

Figure 2: Graph comparing the expression levels of BMP-2 and FGF-2 on day 7 and 14 in control, PMMA-HABBK, and PMMA-HAGMP groups

BMP-2 enhances bone healing by expediting the process, promoting mineralization, facilitating remodeling, and improving biomechanical stiffness. The release of BMP-2 during the initial 4-day period is inadequate to stimulate the differentiation of MSCs. By day 5-6, the presence of BMP-2 is enough to initiate the differentiation of MSCs and promote the formation of bone.[13] This expression of BMP-2 is reported to reach its highest level on day 21.[20] BMP-2 is essential for osteoblast cell growth. The results of the present study indicated an increase in the expression of BMP-2 from day 7 to day 14 in the control group, which is consistent with the findings from previous studies. Moreover, there was a significant increase in expression level of BMP-2 in the PMMA-HA-BBK and PMMA-HA-GMP groups when compared to the control group.

FGF-2 significantly affects bone development, bone production, and fracture healing.[21] FGF-2 accelerates fibroblast cell proliferation, increases angiogenesis, increases BMP-2 expression, and is a marker for osteoblast differentiation, which means increased bone deposits.[22] The MAPK pathway is triggered by FGF-2 signalling and plays a crucial role in regulating the activity of RUNX2, a significant transcription factor involved in bone formation. FGF-2 enhances osteoblast growth and maturation via activating the ERK1/2 pathway, which plays a crucial role in osteoblast differentiation upon sustained release for up to 21 days.[21,23] The findings from this study show that on days 7 and 14, there was an elevated expression of FGF-2 in both the PMMA-HAP-BBK and PMMA-HA-GMP groups compared to the control group.

The results indicate notable differences in BMP-2 and FGF-2 expression between PMMA-HA (BBK) and PMMA-HA (GMP) implants. These variations are influenced by the calcium/phosphorus (Ca/P) ratio within the HA component. In normal calcified bone, the Ca/P ratio increases with increasing calcification.[24] Literature suggests that BBK hydroxyapatite has a Ca/P ratio of approximately 1.64, [25] while HA derived from bovine bone (GMP) typically ranges from 1.696 to 1.911,[26] with a Ca/P molar ratio of 1.33 to 1.67, which is considered ideal for bone formation.[27,28] Differences in the Ca/P ratio between PMMA-HA (BBK) and PMMA-HA (GMP) influence the biomaterial's compatibility and osteoconductivity, affecting its ability to promote bone growth and osseointegration.

Increased Ca^{2+} supports the SMAD pathway's phosphorylation, which directly induces BMP-2 expression. Ca^{2+} ions and BMP-2 work together in the continuous autocrine and paracrine bone formation process.[29] Increasing Ca^{2+} also increases FGF-2 expression.[30] FGF-2 is responsible for BMP-induced nuclear translocation and accumulation of RUNX2, as well as phosphorylation of the SMAD 1/5/8 pathway.[31] This demonstrates that the levels of BMP-2 and FGF-2 expression are elevated in PMMA-HA (GMP) compared to PMMA-HA (BBK).

The findings demonstrated that the PMMA-HA-BBK and PMMA-HA-GMP groups exhibited an enhanced expression of BMP-2 and FGF-2 markers in osteoblast cells when exposed to these two materials. Furthermore, both treatment groups showed a notable elevation in BMP-2 and FGF-2 expression levels compared to the control group.

Conclusion

PMMA-HA combination implants can accelerate osteoblast differentiation, as evidenced by the increase in BMP-2 and FGF-2 as osteogenic biomarkers. These initial findings provide a valuable foundation for subsequent studies to assess the applicability of PMMA-HA implants in dental implant scenarios.

Conflict of Interest

Authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgement

The authors appreciate Mr. I.I. Ogunlowo for his assistance during plant collection.

References

1. Zambonin G, Colucci S, Cantatore F, Grano M. Response of Human Osteoblasts to Polymethylmetacrylate *In Vitro*. Calcif Tissue Int. 1998; 62(4):362-365. doi:10.1007/s002239900445
2. Afiqah IQ, Aziz NAN, Nurhaziqah AMS, Hasiah S. Biocompatible Hydroxyapatite Derive from Selayang Fish Bone Via Mechanochemical Treatment. Solid State Phenom. 2020; 307:339-344. doi:10.4028/WWW.SCIENTIFIC.NET/SSP.307.339
3. Darjanki CM, Prahasanti C, Fitria AE, Kusumawardani B, Wijaksana IKE, Aljunaid M. RUNX2 and ALP Expression in Osteoblast Cells Exposed by PMMA-HAp Combination: An *In Vitro* Study. J Oral Biol Craniofac Res. 2023; 13(2):277. doi:10.1016/J.JOBCR.2023.02.007
4. Saskianti T, Novianti A, Sahar D, Nugraha AP, Putri TS, Kanawa M, Puteri MM, Dewi AM, Ernawati DS. Mixed Polymethylmethacrylate and Hydroxyapatite as a Candidate of Synthetic Graft Materials for Cleft Palate. J Int Dent Med Res. 2022; 15(2):538-543.
5. Saskianti T, Purnamasari S, Pradopo S, Nugraha AP, Prahasanti C, Ernawati DS, Kanawa M. The Effect of Mixed Polymethylmethacrylate and Hydroxyapatite on Viability of Stem Cell from Human Exfoliated Deciduous Teeth and Osteoblast. Eur J Dent. 2023; 18(1):314. doi:10.1055/S-0043-1768971
6. Ayu Pridanti K, Cahyaraeni F, Harijanto E, Rianti D, Kristanto W, Damayanti H, Setiana Putri T, Dinaryanti A, Karsari D, Yuliati A. Characteristics and Cytotoxicity of Hydroxyapatite from Padalarang–Cirebon Limestone as Bone Grafting Candidate. Biochem Cell Arch. 2020; 20(2):4727-4731. Available from: https://connectjournals.com/03896.2020.20.4727
7. Phakatkar AH, Shirdar MR, Qi ML, Taheri MM, Narayanan S, Foroozan T, Sharifi-Asl S, Huang Z, Agrawal M, Lu YP, Shahbazian-Yassar R, Shokuhfar T. Novel PMMA Bone Cement Nanocomposites Containing Magnesium Phosphate Nanosheets and Hydroxyapatite Nanofibers. Mater Sci Eng C Mater Biol Appl. 2020; 109:110497. doi:10.1016/J.MSEC.2019.110497
8. Hienz SA, Paliwal S, Ivanovski S. Mechanisms of Bone Resorption in Periodontitis. J Immunol Res. 2015; 2015:615486. doi:10.1155/2015/615486
9. Farhadieh RD, Gianoutsos MP, Yu Y, Walsh WR. The Role of Bone Morphogenetic Proteins BMP-2 and BMP-4 and Their Related Postreceptor Signaling System (Smads) in Distraction Osteogenesis of the Mandible. J Craniofac Surg. 2004; 15(5):714-718. doi:10.1097/00001665-200409000-00003
10. Murakami S. Periodontal Tissue Regeneration by Signaling Molecule(s): What Role Does Basic Fibroblast Growth Factor (FGF-2) Have in Periodontal Therapy? Periodontol 2000. 2011; 56(107):188-208.
11. Retnam L, Chatikavanij P, Kunjara P, Paramastri YA, Goh YM, Hussein FN, Mutalib AR, Poosala S. Laws, Regulations, Guidelines and Standards for Animal Care and Use for Scientific Purposes in the Countries of Singapore, Thailand, Indonesia, Malaysia, and India. ILAR J. 2016; 57(3):312-323. doi:10.1093/ILAR/ILW038
12. Sheikh Z, Javaid MA, Hamdan N, Hashmi R. Bone Regeneration Using Bone Morphogenetic Proteins and Various Biomaterial Carriers. Materials. 2015; 8(4):1778. doi:10.3390/MA8041778
13. Coracini KF, Fernandes CJ, Barbarini AF, Silva CM, Scabello RT, Oliveira GP, Chadi G. Differential Cellular FGF-2 Upregulation in the Rat Facial Nucleus Following Axotomy, Functional Electrical Stimulation and Corticosterone: A Possible Therapeutic Target to Bell's Palsy. J Brachial Plex Peripher Nerve Inj. 2010; 5(1):16. doi:10.1186/1749-7221-5-16
14. da Silva de Oliveira JC, Luvizuto ER, Sonoda CK, Okamoto R, Garcia-Junior IR. Immunohistochemistry Evaluation of BMP-2 With β-Tricalcium Phosphate Matrix, Polylactic and Polyglycolic Acid Gel, and Calcium Phosphate Cement in Rats. Oral Maxillofac Surg. 2017; 21(2):247-258. doi:10.1007/S10006-017-0624-3
15. Radha G, Balakumar S, Venkatesan B, Vellaichamy E. A Novel Nano-Hydroxyapatite - PMMA Hybrid Scaffolds Adopted by Conjugated Thermal Induced Phase Separation (TIPS) and Wet-Chemical Approach: Analysis of Its Mechanical and Biological Properties. Mater Sci Eng C Mater Biol Appl. 2017; 75:221-228. doi:10.1016/j.msec.2016.12.133
16. Prahasanti C, Krismariono A, Takanamita R, Wijaksana IKE, Suardita K, Saskianti T, Ernawati DS. Enhancement of Osteogenesis Using a Combination of Hydroxyapatite and Stem Cells from Exfoliated Deciduous Teeth. J Int Dent Med Res. 2020; 13(2):508-512.
17. Hao CP, Cao NJ, Zhu YH, Wang W. The Osseointegration and Stability of Dental Implants with Different Surface Treatments in

Animal Models: A Network Meta-Analysis. Sci Rep. 2021; 1-12. doi:10.1038/s41598-021-93307-4
18. Pandey C, Rokaya D, Bhattarai BP. Contemporary Concepts in Osseointegration of Dental Implants: A Review. Biomed Res Int. 2022; 2022:6170452. doi:10.1155/2022/6170452
19. Wu M, Chen G, Li YP. TGF-β and BMP Signaling in Osteoblast, Skeletal Development, and Bone Formation, Homeostasis and Disease. Bone Res. 2016; 4(1):1-21. doi:10.1038/boneres.2016.9
20. Faßbender M, Minkwitz S, Strobel C, Schmidmaier G, Wildemann B. Stimulation of Bone Healing by Sustained Bone Morphogenetic Protein 2 (BMP-2) Delivery. Int J Mol Sci. 2014; 15(5):8539. doi:10.3390/IJMS15058539
21. Novais A, Chatzopoulou E, Chaussain C, Gorin C, Département A hp, Pitié Salpêtrière G, Mondor H, Nord P, Rothschild H. The Potential of FGF-2 in Craniofacial Bone Tissue Engineering: A Review. Cells. 2021. doi:10.3390/cells10040932
22. Carmagnola D, Pellegrini G, Dellavia C, Rimondini L, Varoni E. Tissue Engineering in Periodontology: Biological Mediators for Periodontal Regeneration. Int J Artif Organs. 2019; 42(5):241-257. doi:10.1177/0391398819828558
23. Yun YR, Won JE, Jeon E, Lee S, Kang W, Jo H, Jang JH, Shin US, Kim HW. Fibroblast Growth Factors: Biology, Function, and Application for Tissue Regeneration. J Tissue Eng. 2010; 1(1):1-18. doi:10.4061/2010/218142
24. Hou X, Zhang L, Zhou Z, Luo X, Wang T, Zhao X, Lu B, Chen F, Zheng L. Calcium Phosphate-Based Biomaterials for Bone Repair. J Funct Biomater. 2022; 13(4):187. doi:10.3390/JFB13040187
25. Pridanti KA, Cahyaraeni F, Harijanto E, Soebagijo, Rianti D, Wahyudi Kristanto, Damayanti H, Putri TS, Dinaryanti A, Karsari D, Yuliati A. Characteristics and Cytotoxicity of Hydroxyapatite from Padalarang–Cirebon Limestone as Bone Grafting Candidate. Mater Sci Forum. 2020; 996:4727-4731.
26. Manalu JL, Soegijono B, Indrani DJ. Characterization of Hydroxyapatite Derived from Bovine Bone. Asian J Appl Sci. 2015; 3(4):758-765.
27. Bano N, Salwah Jikan S, Basri H, Adzila Abu Bakar SS, Hussain Nuhu A. Natural Hydroxyapatite Extracted from Bovine Bone. J Sci Technol (*Kota Kinabalu*). 2017; 9(2):22-28.
28. Rianti D, Kristanto W, Damayanti H, Putri TS, Dinaryanti A, Syahrom A, Yuliati A. The Characteristics and Potency of Limestone-Based Carbonate Hydroxyapatite to Viability and Proliferation of Human Umbilical Cord Mesenchymal Stem Cell. Res J Pharm Technol. 2022; 15(5):2285-2292. doi:10.52711/0974-360X.2022.00380
29. Aquino-Martínez R, Artigas N, Gámez B, Rosa JL, Ventura F. Extracellular Calcium Promotes Bone Formation from Bone Marrow Mesenchymal Stem Cells by Amplifying the Effects of BMP-2 on SMAD Signalling. PLoS One. 2017; 12(5):e0178158. doi:10.1371/journal.pone.0178158
30. Kanaya S, Nemoto E, Sakisaka Y, Shimauchi H. Calcium-Mediated Increased Expression of Fibroblast Growth Factor-2 Acts Through NF-κB and PGE2/EP4 Receptor Signaling Pathways in Cementoblasts. Bone. 2013; 56(2):398-405. doi:10.1016/j.bone.2013.06.031
31. Wu M, Chen G, Li YP. TGF-β and BMP Signaling in Osteoblast, Skeletal Development, and Bone Formation, Homeostasis and Disease. Bone Res. 2016; 4(1):1-21. doi:10.1038/boneres.2016.9