ORIGINAL ARTICLE

# Expression of Stro-1, Runx-2, Osterix, and Alp in Alveolar Bone Regeneration Process Following the Administration of Hydroxyapatite Gypsum Puger (HAGP) Scaffold

Amiyatun Naini[1], Agus Sumono[2], Rahardyan Parnaadji[1], Nike Hendrijantini[3], Siti Khaerunnisa[4]

[1] Departement of Prostodontic, Faculty of Dentistry, University of Jember, Jember-Indonesia.
[2] Departement of Basic Dentistry, Faculty of Dentistry, University of Jember, Jember-Indonesia
[3] Departement of Prostodontic, Faculty of Dentistry, Airlangga University, Surabaya- Indonesia.
[4] Departement of Physiology and Medical Biochemistry , Faculty of Medicine, Airlangga University, Surabaya- Indonesia.

**ABSTRACT**

**Introduction:** Tooth extraction before denture placement could result in trauma and damage to up to 50% of the alveolar bone, inducing bone resorption, and affecting the patient's quality of life. Hydroxyapatite Gypsum Puger (HAGP) can be used as an alternative to bone graft material which degrades slowly, affecting the proliferation and activity of cells that are responsible for bone tissue engineering. This study aimed to analyze the regeneration mechanism of alveolar bone by administering the HAGP scaffold and observing the Stro-1, Runx-2, Osterix, and ALP expression. **Methods:** Laboratory experimental research was conducted and we used 150-355µm HAGP scaffold particles, applied in vivo inside alveolar sockets of the rats for 7, 14, and 28 days, followed by immunohistochemical examination of Stro-1, Runx-2, Osterix, and ALP expressions. **Results:** The HAGP scaffold group showed that the Stro-1 expression was significantly higher than the K(-) group, and the Runx-2 expression increased on day 7 and decreased on day 28 in the HAGP and K(-) groups. Osterix expression increased from day 7, 14, to day 28. The high expression of Osterix on day 28 means it took over the Runx-2 function. In ALP there was a significant increase on day 7. ALP expression was a sign of early osteoblast differentiation and production by cells, this extracellular matrix mineralization is an indicator of the osteogenic process. **Conclusion:** Alveolar bone regeneration mechanism in rats revealed that the expression of Stro-1, Runx-2, Osterix, and ALP was higher in the HAGP scaffold group compared to the control group on days 7,14, and 28.
*Malaysian Journal of Medicine and Health Sciences* (2023) 19(3):145-151. doi:10.47836/mjmhs19.3.19

**Keywords:** Stro-1, Runx-2, Osterix, Alp, Alveolar Bone Regeneration, Hydroxyapatite Gypsum Puger Scaffold

**Corresponding Author:**
Amiyatun Naini, PhD
Email: amiyatunnaini.fkg@unej.ac.id
Tel: +6289622144167

## INTRODUCTION

Tooth extraction before denture placement can result in extraction-related trauma and damage to the alveolar bone by more than 50%. Physiologically, alveolar bone resorption will occur following tooth extraction (1). Furthermore, the effect of alveolar bone resorption will affect the success rate of dentures, and usage hence in turn affecting the quality of life of a person. Some literature stated that many patients experience various problems related to chewing function, difficulty speaking, and poor aesthetics due to the failure of denture placement associated with the resorption of the alveolar bone (2,3).

In the scope of Prosthodontics, alveolar ridge resorption turns out to be a major problem for dentists because it affects the support, retention, stabilization, and masticatory function of denture users. The incidence of alveolar ridge resorption will be more pronounced if the socket of the alveolar ridge from the tooth extraction is not immediately preserved using graft material (4).

Based on such a phenomenon, it is necessary to address post-tooth extraction before denture placement. One of the promising graft materials for preservation is hydroxyapatite from Gypsum Puger. Gypsum Puger has been successfully synthesized into hydroxyapatite powder (5). HAGP powder has been successfully synthesized into HAGP scaffold with 100% purity level of hydroxyapatite by XRD test, SEM examination found abundant pores on the scaffold with an average size of 30 µm that the results are the same or there is a pattern similar to the HAB scaffold (the gold standard). This material degrades slowly based on the HAGP scaffold degradation test. This will affect the cellular proliferation and activity so that it enters and grows

into the scaffold which helps with tissue engineering. Imported scaffolding materials are relatively expensive, so this HAGP, which comes from abundant natural materials in Indonesia, can be used as a high-quality material with bone composition. However, this HAGP has a brittle weakness, therefore if further research is carried out, it can be added with biopolymer material to increase its mechanical strength (6).

In other studies related to bone regeneration mechanisms, it is stated that the differentiation of osteoblasts into bone cells is regulated by transcription factors such as Stro-1, Runx2, Osterix, and Alkaline Phosphatase (ALP) (7). Stro-1 is a cell surface antigen expressed by stromal elements in the human bone marrow. A subset of STRO-1 positive enriched marrow cells was able to differentiate several mesenchymal lineages including stromal cells that support hematopoiesis with a phenotype in osteoblasts. Runx2 is a master gene that has a prominent role in the process of differentiation of mesenchymal cells into osteoblasts as cells for alveolar bone regeneration, osterix in bone regeneration for evaluation of osteoblast maturation so that it can show osteocyte formation, ALP is the main protein of the bone matrix. Active osteoblast cells produce osteoid tissue and secrete large amounts of alkaline phosphatase, which plays an important role in depositing calcium and phosphate into the bone matrix (8–10). The research objective was to analyze the expression of stro-1, runx-2, osterix, and ALP in the regeneration process of alveolar bone after administration of the Hydroxyapatite Gypsum Puger scaffold.

## MATERIALS AND METHODS

### Preparation of experimental animals

Before conducting the research, approval was obtained from the ethical feasibility team of the Faculty of Dentistry, the University of Jember, certificate No. 993 / UN.25.8 / KEPK / DL / 2020. Samples used lower jaws were carried out the removal of the first molar teeth from 36 rats with the following criteria: age 12-14 weeks, males, body weight about 200-250 grams, standard feeding and drinking water ad libitum, maintenance of animals.

### HAGP scaffold preparation

The manufacture of HAGP scaffold material, namely gypsum powder from puger, was sieved with a particle size of <50μm. Weighing DHP with a mechanical balance to make a solution with a concentration of 0.5 M. Weighing gypsum powder to be mixed with the solution, in a ratio of 5 g of powder and 400 ml of DHP solution. The solution is then put into the microwave and heated (hydrothermal process) at a temperature of 100 0C for 30 minutes. The solution was then washed using distilled water and at the same time filtered using filter paper several times until the pH was neutral. Then the powder was dried in a microwave at 500C for 5 hours. Hydroxyapatite weighed 4 g mixed with liquid gelatin. Solid gelatin is melted with hot water at a temperature of 60 0C to become 10% liquid gelatin. Then 4 g of Hydroxyapatite mixed with liquid gelatin up to 10 ml were frozen and dried in a sublimation/freeze-dried system. Then crushed and sieved with a particle size of 150-355 m. Then the Gamma radiation was sterilized at BATAN. Then the preparation of Scaffold HAGP concentration of 10% by mixing 0.05 grams of scaffold HAGP and 1.95 grams of Polyethylene Glycol (PEG). PEG is produced by mixing 3.92 grams of PEG 400 (solid) with 0.98 grams of PEG 4000 (liquid) (11).

### HAGP scaffold applications

A total of 36 male adult Wistar rats were divided into 9 groups, namely the normal control group for 7 days (KN7), normal control for 14 days (KN14), normal control for 28 days (KN28), negative control group for 7 days (K(-)7), negative control group for 14 days (K(-)14), the negative control group for 28 days (K(-)28, HAGP group for 7 days (HAGP7), HAGP group for 14 days (HAGP14), HAGP group for 28 days (HAGP28).

Wistar rats were anesthetized intramuscularly using ketamine 100mg/ml and xylazine base 20mg/ml in 1:1 ratio with a dose of 0.08-0.2 ml/kg BW. After the rats were anesthetized, the mandibular left first molar was extracted using a needle holder. Then the application of 10% HAGP scaffold as much as 0.1 g into the extraction socket is then sewn. Then preservation is done by inserting the HAGP scaffold material into the extraction socket and then suturing it. The observation was placed for 7, 14, and 28 days. The rats were sacrificed, and we carefully cut the left mandible from anterior to posterior and washed it with PBS. The alveolar bone demineralization was carried out using 15% EDTA solution for 4-6 weeks (the solution was renewed every 3 days).

### Immunohistochemical staining of Stro-1, Runx-2, Osterix, and ALP

After that, we proceed to create paraffin block preparation and Immunohistochemically stained it for Stro-1, Runx-2, Osterix, and ALP by locking the slide with PBS Ph 7.4 once for 5 minutes. Blocking endogenous peroxide, wash water, then PBS, enzymes, wash PBS again, and then add anti-rat mouse antibodies. Blocking unspecific protein using 5% FBS containing 0.25% Triton X-10 and washing using PBS pH 7.4 three times for 5 minutes. Incubation using anti-mouse anti-rat to connect anti-mouse then given anti-mouse biotinylated label, after that washing PBS was given streptafubi conjugated with HRP, then washing and then given substrate (peroxidase and chromogen) then washing was given hematoxylin washing, dehydration covered with cover glass. Lastly, the examination was performed with a light microscope.

### Statistical analysis

The data obtained were subjected to statistical analysis

with a Statistical Package for the Social Sciences (SPSS) software, version 22. All scale variables were analyzed for normality and homogeneity tests. If the data were normally distributed, there was a need to conduct parametric tests using One Way ANOVA followed by Least Significant Difference (LSD). The $p < 0,05$ indicated statistical significance.

## RESULTS

Examination results of Runx2, Osterix, and ALP Stro1 Expressions in the Normal Control (KN), Negative Control (K -), Hydroxyapatite Gypsum Puger (HAGP) scaffold on days 7, 14, and 28 are shown in Figure 1.

The results of the stro1 expression data were analyzed using the Shapiro Wilk normality test and the p>0.05 result indicates that the data were normally distributed. Then the homogeneity test with the Levene test was carried out, resulting in p = 0.04 which means that the data variance was not homogeneous among the groups, then we continued with the Brown-Forsythe test and obtained p = 0.000, which means there is a difference in stro1 between groups (Table I). After that, the multiple comparisons Games-Howell test was performed to find out which group pairs are different. From the analysis, there were significant differences between groups KN7 and HAGP14 (p = 0.001), between groups KN14 and HAGP7 (p = 0.030), between groups KN14 and HAGP14 (p = 0.034 ), between groups KN28 and HAGP7 (p = 0.037 ), between the KN8 and HAGP14 groups (p=0.002). Details of the Stro-1 expression can be seen in figure 2.

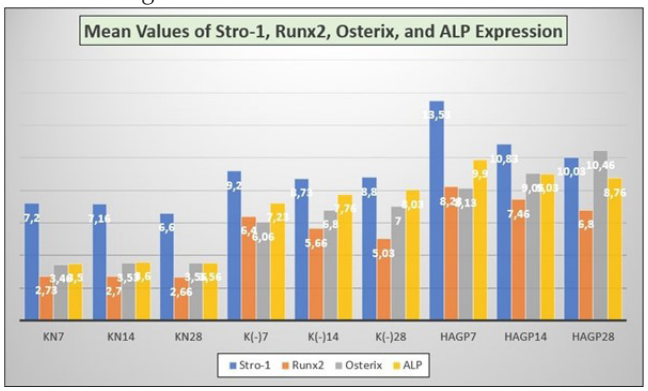


**Figure 1: Average expression of Stro-1, Runx2, Osterix, and ALP in the HAGP7 group which was the highest followed by HAGP14, HAGP28, K (-) 7, K (-) 28, K (-) 14, KN7, KN14, KN28.**

**Table I: Description of the Stro1, Runx2, Osterix and ALP expression data among the treatment groups**

| Group | Stro1 | | Runx2 | | Osterix | | ALP | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| KN 7 | 7.200 | 0.200 | 2.733 | 0.153 | 3.467 | 0.416 | 3.500 | 0.755 |
| KN 14 | 7.167 | 0.681 | 2.700 | 0.400 | 3.533 | 0.153 | 3.600 | 0.300 |
| KN 28 | 6.600 | 0.400 | 2.667 | 0.208 | 3.567 | 0.208 | 3.567 | 0.153 |
| K(-)7 | 9.200 | 1.652 | 6.400 | 0.265 | 6.067 | 0.115 | 7.233 | 0.513 |
| K(-) 14 | 8.733 | 0.839 | 5.667 | 0.252 | 6.800 | 0.265 | 7.767 | 0.551 |
| K(-) 28 | 8.800 | 0.854 | 5.033 | 0.306 | 7.000 | 0.100 | 8.033 | 0.513 |
| HAGP 7 | 13.533 | 1.266 | 8.233 | 0.153 | 8.133 | 0.321 | 9.900 | 0.173 |
| HAGP 14 | 10.833 | 0.252 | 7.467 | 0.503 | 9.067 | 0.153 | 9.033 | 0.351 |
| HAGP 28 | 10.033 | 0.850 | 6.800 | 0.400 | 10.467 | 0.643 | 8.767 | 0.611 |

Note : Significant at α=0.05

### Runx2 Expression Results

The results of the Runx2 expression data were analyzed using the Shapiro Wilk normality test, which was found to be p> 0.05, this indicates that the data were normally distributed. After that, the homogeneity test with the Levene test was performed with a score of p = 0.652, which means that the variance of the data was homogeneous between groups. Then ANOVA test was carried out with the result of p = 0.000, which means that there are differences in Stro1 among the groups. Lastly, we did multiple comparisons with LSD to find out which group pairs are different. The results of the different test using LSD analysis showed that the Runx-2 expression was significantly different, namely between groups K(-) 7 and HAGP 7 (p= 0.000), between groups K(-) 14 and HAGP 7 (p= 0.000), between groups K (-) 28 and HAGP 7 (p=0.000), between groups HAGP 14 and HAGP 7 (p=0.008), between groups HAGP 28 and HAGP 7 (p=0.000), between groups K(-) 7 and HAGP 14 ( p=0.001) , between groups K(-) 14 and HAGP 14 (p=0.001), between groups K(-) 28 and HAGP 14 (p=0.000), between K(-) 28 and HAGP 14 (p=0.000), between groups HAGP 28 and HAGP 14 (p=0.018), between groups K(-)14 and HAGP 28 (p=0.000), between groups K(-) 28 and HAGP 28 (p=0.000), this indicates that the treatment on the 7th day of expression increased and the longer the treatment the Runx-2 expression decreased, namely in the HAGP group on day 28, in accordance with other studies that research conducted with HA preservation on day 7 can increase the expression of Runx-2 which regulates MSCs to differentiate into osteoprogenitor and preosteoblast directions. both, directly and indirectly, regulate the

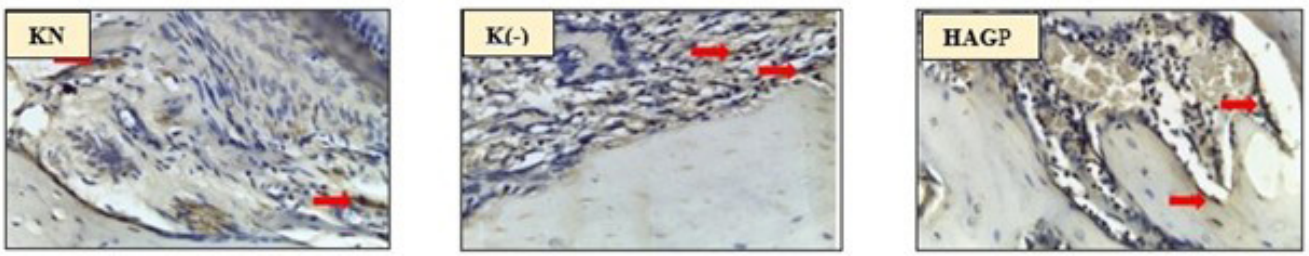


**Figure 2: The immunohistochemical examination of Stro-1 expression is shown by the arrow.** There appears to be a difference between the treatment groups, KN, K(-), and HAGP. (IHC staining. 400x magnification; Nikon H600L microscope; 300 megapixels DS Fi2 camera)

number of other specific genes for osterix and type 1 collagen (12,13). Meanwhile, on day 28, the expression of Runx-2 between HAGP and control was significantly different (Figure 3).

**Osterix Expression Results**

The results of the Osterix expression data were analyzed using the Shapiro Wilk normality test and the value was p>0.05, this indicates that the data were normally distributed. Then we conducted a homogeneity test using the Levene test with a score of p=0.009 which means that the data variance was not homogeneous between groups. After that, we performed the Brown-Forsythe test with the result of p=0.000, which means there is a difference in Stro1 among the groups, followed by multiple comparisons Games-Howell to find out which group pairs are different. The results of the different tests with Games-Howell analysis showed that there was a significant difference in Osterix expression between groups K(-)7 and HAGP 7 (p=0.023), between groups K(-) 7 and HAGP 14 (p=0.000), between groups K (-) 7 and HAGP 28 (p= 0.029), between groups K(-) 14 and HAGP 7 (p= 0.048), between groups K(-) 14 and HAGP 28 (p=0.027), between groups K(- ) 28 and HAGP 14 (p=0.001), between groups K(-)14 and HAGP 28 (p=0.027), this means that between HAGP and K(-) can be said to be not the same in Osterix expression, because in group K(- ) rats that have been extracted but not given scaffold material. Microscopic examination of osterix expression can be seen in Figure 4.

**Alkaline Phosphatase (ALP) Expression Result**

The results of the ALP expression data were analyzed using the Shapiro Wilk normality test, it was then obtained p> 0.05 which indicates that the data were normally distributed. We followed by the homogeneity test with the Levene test obtained p = 0.263 meaning that the variance of the data was homogeneous between groups, then we proceed with the Anova test, and it obtained p = 0.000 which means that there are differences in ALP between groups. Lastly, we did multiple comparisons with LSD to find out which pairs of groups are different. The LSD analysis revealed that there was no difference in ALP expression between the KN7, KN14, and KN28 groups, because in the normal control group (KN) on days 7, 14, and 28, no tooth extraction was performed thus no preservation was carried out so the expression was not different, K (-) 28 and HAGP 28 were not different either. However, in the K (-) 28 group, there was an increase in expression, whereas in the HAGP28 group there had been a decrease in ALP expression from day 7 and the numbers for both groups were nearly identical. Meanwhile, the other groups differed significantly on days 7, 14, and 28. The ALP expression is shown in Figure 5.

**DISCUSSION**

The expression of strong-1 was significantly higher in the HAGP group compared to the K(-) group. Stro1 is an early marker for cells in the osteogenic differentiation process. In vitro research stated that the expression of stro1 increased on day 7 and day 14, then declined steadily on day 28 (14,15). Stromal precursor antigen-1 is a marker for MSCs. Stro-1 is considered an early marker of MSCs, stromal precursor cells, and characteristic of progenitor cells (16,17).

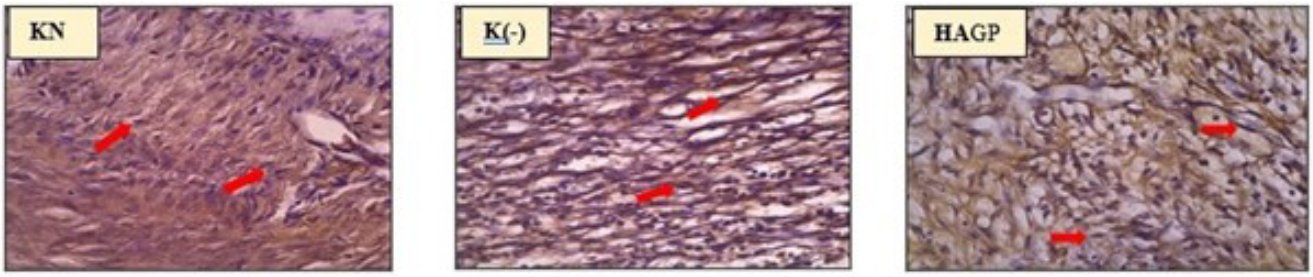


**Figure 3: Immunohistochemical examination revealed Runx-2 expression, shown by the arrow.** There is a noticeable difference between the treatment groups, Runx-2 expression in the KN, K(-), and HAGP groups (IHC stain, 400x magnification; Nikon H600L microscope; 300 megapixels DS Fi2 camera)

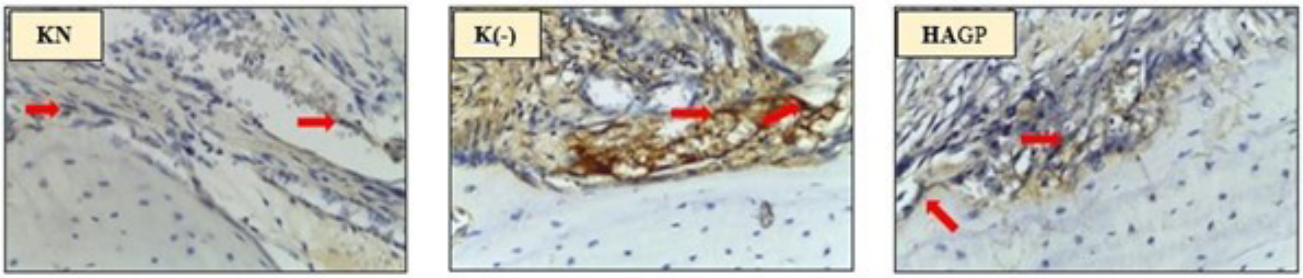


**Figure 4: Immunohistochemical examination results of Osterix expression are shown by the arrow.** There appears to be a difference between the treatment groups, KN, K (-), and HAGP. (IHC stain. 400x magnification; Nikon H600L microscope; 300 megapixels DS Fi2 camera)

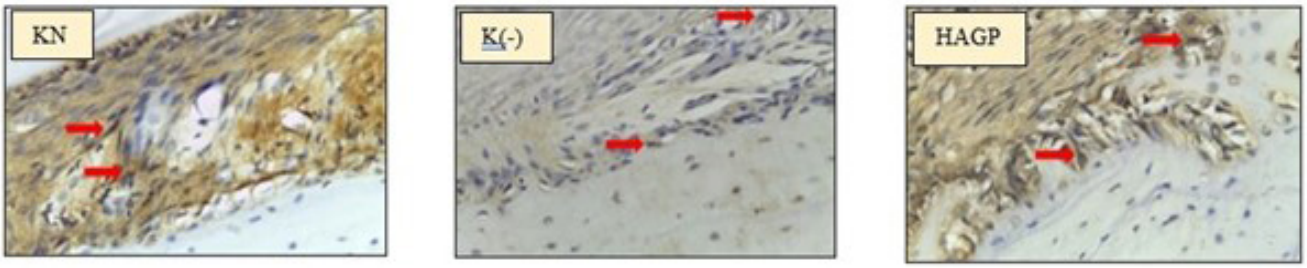


**Figure 5: Immunohistochemical examination results of ALP expression are shown by arrows.** It can be seen that there is a difference between the treatment groups, KN, K (-), and HAGP. (IHC staining. 400x magnification; Nikon H600L microscope; 300 megapixels DS Fi2 camera)

In this study, Runx-2 expression increased on day 7 and decreased on day 28 in the HAGP and K (-) groups that indicating that Runx-2 is the main transcription factor in osteogenic differentiation in MSCs (12,17), and a surge of Runx-2 expression on day 7 was due to differentiation of MSCs into preosteoblasts or immature osteoblasts, and on day 28 there was a decrease in Runx-2 expression due to the occurrence of mature osteoblast processes (18).

The expression of osterix in the HAGP group means that osteoblast maturation happened faster in the HAGP group, to previous studies that the expression of osterix is regulated and required during osteoblast maturation (18). The high osterix expression was in line with the lower Runx-2 expression in the HAGP group on day 28 because osterix was also an important transcription factor and was not downstream to Runx-2 (19). Osterix expression that was found higher in HAGP compared to K(-) indicated that osteoblasts matured in HAGP to form osteocytes were faster than in K(-). According to table 1. in this study, it was found that the Osterix expression increased from day 7, 14 to day 28. The high expression of Osterix on day 28 seems to replace the Runx-2 function because on day 28 the Runx-2 expression decreases when compared to day 7 (20,21).

In the ALP expression the longer the treatment took the lower the expression, it can be seen in the HAGP group on day 28, this is by other studies that there was a significant increase in ALP on day 7, ALP expression could be a sign of early osteoblast differentiation and production. This indicates the presence of extracellular matrix mineralization which is an indication of osteogenic (17,22,23). The ALP expression assessment is performed to assess the osteoblastic differentiation process in bone tissue (24).

Briefly, the analysis for bone regeneration associated with the HAGP 7, HAB 7 and HAB 28 groups showed a significant pathway to Runx-2 resulting in the formation of the trabecular bone area, whereas in group 28 there was a significant pathway analysis to Runx-2, osterix until the formation of broad bone trabeculae. At high Osterix it will cause ossification and further maturation will occur in the bone that is still in the process of walking.

Increased bone mass in the alveolar ridge socket as the main protein which is the largest composition in the bone matrix that has a pathway to the area of bone trabeculae. Path analysis in the HAGP 28 group has a mechanism for a faster bone regeneration process than the other groups so that the HAGP scaffold can be used as an alternative as a scaffold base material for bone socket regeneration.

The process of bone regeneration will occur faster if the addition of bone graft material. The mechanism of bone regeneration is the presence of bone grafts that are preserved in bone tissue and in that tissue, there are also endogenous MSCs which are characterized by the increased stro-1 expression on day 7 and day 14 then differentiation proliferation will occur to stimulate osteoprogenitor cells to become osteoblasts. Osteoblasts transcribe Runx 2 and osterix. Osteoblasts can secrete alkaline phosphatase then mineralization and ossification will occur so that bone trabeculae are formed or bone regeneration occurs (25,26).

## CONCLUSION

In the mechanism of alveolar bone regeneration in rats, the expression of Stro-1, Runx-2, Osterix, and ALP were higher in the HAGP group than in the control group on days 7,14, and 28. Limitations of research conducted in vivo need to be extended to further studies in large animals to alveolar bone remodeling which requires the formation of old bone. While this research is a maximum of 28 days, even though the bone regeneration process takes a longer time. Further experimental research is needed to assess a more perfect bone regeneration process. The future perspective of this material will form a commercial product.

## ACKNOWLEDGEMENTS

The authors would like to thank the research grant supporting the IDB program at the University of Jember in 2020.

**REFERENCES**


1. Pelegrine AA, Da Costa CES, Correa MEP, Marques JFC. Clinical and histomorphometric evaluation of extraction sockets treated with an autologous bone marrow graft. Clin Oral Implants Res. 2010;21(5):535–42. doi: 10.1111/j.1600-0501.2009.01891.x
2. Puspitadewi SR. PERAWATAN PROSTHODONTIK PADA KONDISI RIDGE YANG KURANG MENGUNTUNGKAN. 2015;2(2):133–42.
3. Babu B, Dinesh ; Jain, Veena ; Pruthi, Gunjan ; Mangtani, Nidhi ; Pillai RS. Effect of denture soft liner on mandibular ridge resorption in complete denture wearers after 6 and 12 months of denture insertion: A prospective randomized clinical study. J Indian Prosthodont Soc. 2017;17(3):233–8. doi: 10.4103/jips.jips_113_17
4. O'Keefe RJ, Jacobs JJ, Chu CR, Einhorn TA. Orthopaedic basic science: Foundations of clinical practice, fourth edition. Wolters Kluwer Health; 2018. 371 p.
5. Naini A, Ardhiyanto HB, Yustisia Y. Proses sintesis dan karakterisasi hydoxyapatite menggunakan analisis XRD FTIR dari gypsum puger Kabupaten Jember sebagai material augmentasi ridge alveolar. Stomatognatic (JKG Unej). 2014;11(2):32–7.
6. Naini, A., Sudiana, I.K., Rubianto, M., Mahyudin, F., Mufti N. Characterization and degradation of hydroxyapatite gypsum puger (hagp) freeze dried scaffold as a graft material for preservation of the alveolar bone socket. J Int Dent Med Ressearch. 2018;11(2):532–6.
7. Komori T. Regulation of osteoblast differentiation by transcription factors. J Cell Biochem. 2006;99(5):1233–9. doi: 10.1002/jcb.20958.
8. Bishop, Michael L ; Fody, Edward P; Schoeff L. Clinical Chemistry: Techniques, Principles, Correlations (Bishop, Clinical Chemistry) 6th Edition. 6th ed. Philadelphia : Wolters Kluwer/ Lippincott Williams & Wilkins; 2010. 732 p.
9. Cardemi C, Elgali I, Xia W, Emanuelsson L, Norlindh B, Omar O, et al. Strontium-doped calcium phosphate and hydroxyapatite granules promote different inflammatory and bone remodelling responses in normal and ovariectomised rats. PLoS One. 2013;8(12):1–17. doi: 10.1371/journal.pone.0084932.
10. Vieira AE, Repeke CE, De Barros Ferreira S, Colavite PM, Biguetti CC, Oliveira RC, et al. Intramembranous bone healing process subsequent to tooth extraction in mice: Micro-computed tomography, histomorphometric and molecular characterization. PLoS One. 2015;10(5):1–22. doi: 10.1371/journal.pone.0128021.
11. Naini A, Sudiana IK, Rubianto M, Kresnoadi U, Latief FDE. Effects of hydroxyapatite gypsum puger scaffold applied to rat alveolar bone sockets on osteoclasts, osteoblasts and the trabecular bone area. Dent J (Majalah Kedokt Gigi). 2019;52(1):13. doi: 10.20473/J.DJMKG.V52.I1.P13-17
12. Bostrum KI, Rajamannan NM, Towler DA. The regulation of valvular and vascular sclerosis by osteogenic morphogens. Circ Res. 2011;109(5):564–77. doi: 10.1161/CIRCRESAHA.110.234278.
13. Geng Z, Yuan Q, Zhuo X, Li Z, Cui Z, Zhu S, et al. Synthesis, characterization, and biological evaluation of nanostructured hydroxyapatite with different dimensions. Nanomaterials. 2017;7(2):1–13. doi: 10.3390/nano7020038
14. Byers RJ, Brown J, Brandwood C, Wood P, Staley W, Hainey L, et al. Osteoblastic differentiation and mRNA analysis of STRO-1-positive human bone marrow stromal cells using primary in vitro culture and poly (A) PCR. J Pathol. 1999;187(3):374–81. doi: 10.1002/(SICI)1096-9896(199902)187:3<374::AID-PATH257>3.0.CO;2-V.
15. Ercal P, Pekozer GG, Gumru OZ, Kose GT, Ramazanoglu M. Influence of STRO-1 selection on osteogenic potential of human tooth germ derived mesenchymal stem cells. Arch Oral Biol. 2017;82(March):293–301. doi: 10.1016/j.archoralbio.2017.06.028
16. Psaltis PJ, Paton S, See F, Arthur A, Martin S, Itescu S, et al. Enrichment for STRO-1 expression enhances the cardiovascular paracrine activity of human bone marrow-derived mesenchymal cell populations. J Cell Physiol. 2010;223(2):530–40. doi: 10.1002/jcp.22081.
17. Liu X, He W, Fang Z, Kienzle A, Feng Q. Influence of silver nanoparticles on osteogenic differentiation of human mesenchymal stem cells. J Biomed Nanotechnol. 2014;10(7):1277–85. doi: 10.1166/jbn.2014.1824.
18. Chen Q, Shou P, Zheng C, Jiang M, Cao G, Yang Q, et al. Fate decision of mesenchymal stem cells: Adipocytes or osteoblasts? Cell Death Differ. 2016;23(7):1128–39. doi: 10.1038/cdd.2015.168.
19. Bear M, Butcher M, Shaughnessy SG. Oxidized low-density lipoprotein acts synergistically with β-glycerophosphate to induce osteoblast differentiation in primary cultures of vascular smooth muscle cells. J Cell Biochem. 2008;105(1):185–93. doi: 10.1002/jcb.21812.
20. Wang C, Liao H, Cao Z. Role of osterix and microRNAs in bone formation and tooth development. Med Sci Monit. 2016;22:2934–42. doi: 10.12659/msm.896742.
21. Fu X, Li Y, Huang T, Yu Z, Ma K, Yang M, et al. Runx2/Osterix and Zinc Uptake Synergize to Orchestrate Osteogenic Differentiation and Citrate Containing Bone Apatite Formation. Adv Sci. 2018;5(4). doi: 10.1002/advs.201700755.
22. Uskoković V, Hoover C, Vukomanović M, Uskoković DP, Desai TA. Osteogenic and antimicrobial nanoparticulate calcium phosphate and poly-(d,l-lactide-co-glycolide) powders for the treatment of

osteomyelitis. Mater Sci Eng C. 2013;33(6):3362–73. doi: 10.1016/j.msec.2013.04.023.
23. Jin X, Chen X, Cheng Y, Wang L, Hu B, Tan J. Effects of hydrothermal temperature and time on hydrothermal synthesis of colloidal hydroxyapatite nanorods in the presence of sodium citrate. J Colloid Interface Sci. 2015;450:151–8. doi: 10.1016/j.jcis.2015.03.010.
24. Hanna H, Mir LM, Andre FM. In vitro osteoblastic differentiation of mesenchymal stem cells generates cell layers with distinct properties. Stem Cell Res Ther. 2018;9(1):1–11. doi: 10.1186/s13287-018-0942-x.
25. Lv FJ, Tuan RS, Cheung KMC, Leung VYL. Concise review: The surface markers and identity of human mesenchymal stem cells. Stem Cells. 2014;32(6):1408–19. doi: 10.1002/stem.1681.
26. Chen Y, Xiong M, Dong Y, Haberman A, Cao J, Liu H, et al. Chemical Control of Grafted Human PSC-Derived Neurons in a Mouse Model of Parkinson's Disease. Cell Stem Cell. 2016;18(6):817–26. doi: 10.1016/j.stem.2016.03.014.