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The Effects of Putty and Granule Beta-tricalcium Phosphate on Bone Cell Histomorphometry and Ki67 Expression in Sheep Tibia

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Abstract

THE TIME period for healing bone fractures in humans and animals is extremely important. This study aimed to compare the effect of tricalcium phosphate putty and granules on bone defect healing in sheep. The study included 4 male sheep. Each animal underwent tibia surgery by creating three defects in the same tibia, the first to apply the granule material, the second to control, and the third to apply the putty material, with an observation period of 2-8 weeks. Significant differences were observed in the number of osteocytes between the granule groups and the putty group compared to the control in the different period. Both forms showed an increase in the number of osteocytes. A significant decrease was recorded in the number of osteoblast cells in the putty group after 8 weeks of treatment. It was found that the speed of resorption of the putty material was faster in the putty form than in the granules, and the speed of the bone's response to healing was faster during the same period. The granule group displayed consistently moderate Ki67 expression at 2, 4, 6, and 8 weeks, and conversely, the putty group showed intense Ki67 expression at 4 weeks and 8 weeks. Both forms showed a good response to the healing of the bone defect, although. The putty form of β-TCP was better, had faster resorption after 8 weeks of treatment compared to the granular form.

Keywords: Tibia, Osteocyte, Osteoblasts, Sheep.

Introduction

Bone grafting is a dental technique used in various dental treatments like dental implants, ridge augmentation, sinus lift, socket preservation, and periodontal therapy [1]. The ability of bone tissue to regenerate allows for easy repair of abnormalities or fractures, but in severe circumstances, these pathways are restricted [2]. Massive bone deficiencies can be caused by malignant tumors and severe trauma, necessitating a variety of treatments such as the induced membrane technique, allogenic bone grafting, synthetic bone grafting, artificial joint replacement, and autologous bone grafting [3].

The critical size defect (CSD) is an experimental approach used in preclinical orthopedics and trauma surgery to assess biomaterials' efficiency in inducing bone regeneration [4].The best bone transplant material is determined by aspects such as availability, defect size, graft size, form, biomechanics, handling, cost, ethical concerns, biological properties, and associated consequences [5].

Autogenous, allograft, xenograft, and synthetic biomaterials are among the bone regeneration options. Autogenous bone is still the gold standard in the field of bone regeneration due to its osteoinductive and osteogenic properties [6]. Allografts, xenografts and alloplasts have been produced to obviate these drawbacks of autogenous grafts. Synthetic bone grafts have been shown to produce greater clinical bone defect fill as a bone graft substitute [7]

Calcium sulfate, tricalcium phosphate, and coralline hydroxyapatite are examples of alloplastic graft materials with osteoconductive characteristics [8]. Because of their adaptability to bone defect morphology, injectable materials such as putties, cements, puttys, and gels have inspired interest in bone regeneration [9] .

Injectable dental putties in syringes are a Beta Tri-calcium-phosphosilicate bone graft material with improved handling properties. It is made of bioactive glass and contains additives such as HPMC and glycerin, and it is absorbed upon implantation for tissue penetration [10]. Putty,

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which was approved for dental applications in 2007, is a bioactive regenerative material that uses calcium phosphosilicate putty as an osteoconductive scaffold and interacts with adjacent tissues to reduce graft waste and chair-side time [10]

The purpose of this study is to compare the effect of -tri-calcium phosphate Putty and granules on the healing of bone deformities in sheep.

Material and Methods

Chemicals and medicine

POWERBONE® offers an injectable and formable putty bone graft using β-TCP granules or putty, including ZrO_2 particles, for antibacterial efficacy.

Animals

Four healthy male sheep between the ages of 1-1.5 years weighting (40-50 kg) were researched, overseen by veterinarians, acclimated for two weeks, and disease-inspected. At a steady temperature, all procedures were conducted by the same surgeon.

Approval and Location of Study

The Scientific Research Committee and the Department of Oral and Maxillofacial Surgery approved the study, which will be undertaken at the University of Mosul's College of Veterinary in 2021/2023.

Study design

Four healthy male sheep aged 1-1.5 years were used in the investigation, with four experimental periods of 2, 4, 6, and 8 weeks. Each sheep underwent tibia surgery and was monitored for eight weeks. The tibia was treated to three defects: one with tricalcium phosphate granules, one with a negative control group, and one with tricalcium phosphate putty. After an 8-week period, the animals were slaughtered to collect the treated tibia bone.

Micromorphometric Measurements

All parameters were measured using the color USB 2.0 digital image camera (Omax ToupView 9.0-Megapexil China) which was provided with image processing software. The software of camera was calibrated to all lenses of Microscope-Olympus-CX31 by aid of 0.01mm stage micrometer (ESM-11 / Japan).

Statically analysis

The histomorphometrical analysis was carried out using a computer package (Sigma Stat V12.0 / SYSTAT software). Data were presented as means SE (standard error) and analyzed using the One Way ANOVA test with Duncan's test at a significant threshold of P0.05. The non-parametric data of Ki67 immunohistochemical scores were evaluated as median and IQR (Inter-Quartile-Range) using the Kruskal-Wallis test and the Tukey Test, with a significant level set at P0.05.

Results

The table 1 and (Fig 2) presents the means of Osteocyte numbers in different groups and time periods, along with the associated p-values for testing significant differences. Table 1 presents three groups (Control, Granules, Putty) and four time periods, representing the mean number of Osteocytes per 40x field for each group and period.

Significant differences were observed among the material groups (Control, Granules, and Putty) at the corresponding time period, as indicated by a p-value (<0.001). there are Significant differences are observed among time periods within the Control, Granules, and Putty groups, with capital letters indicating different periods, such as "A" for 2-week, "B" for 4-week, "C" for 6-week, and "D" for 8-week periods. The analysis shows significant differences in Osteocyte count between material groups (Control, Granules, Putty) and time periods, with the type of material and time period significantly influencing this variable. Researchers can interpret data to understand the impact of materials and time on osteocyte number, with "Granules" group showing significant differences at 2 weeks.

This Table 2 and Figure 3 presents three groups (Control, Granules, Putty) and four time periods (2w, 4w, 6w, 8w), representing the mean number of Osteoblasts per 40x field. The result recorded Significant differences observed among the material groups (Control, Granules, and Putty) at the corresponding time period, as indicated by the p-value (<0.001). Significant differences are observed among time periods within the Control, Granules, and Putty groups, with p-values indicating different durations for each group. The analysis shows significant differences in the number of Osteoblasts between material groups (Control, Granules, Putty) and time periods, as indicated by the p-values.

The table reveals variations in Osteoblast numbers across material groups and time periods, with p-values confirming statistical significance, aiding researchers in understanding the impact of materials and time.

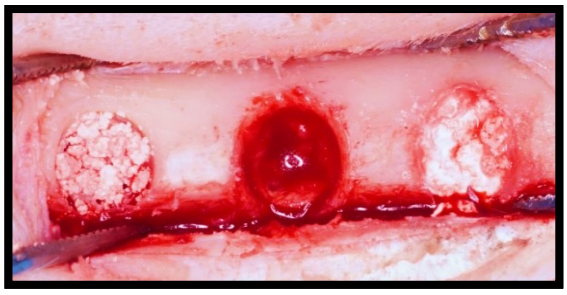

Fig.1. A-Defect filled with Beta tri-calcium phosphate Granule, B-Defect left empty for physiological clot to fill, C-Defect filled with Beta tri-calcium phosphate Putty.

TABLE 1. The means of the Osteocytes numbers in all groups and periods

	Osteocyte No./40x field Mean ± SE			
	Control	**Granules**	**Putty**	***P*-Value**
2w	8.400 ± 0.51^{aA}	32.7 ± 1.31^{bA}	36.40 ± 3.78^{cA}	<0.001
4w	28.2 ± 1.28^{aB}	49.2 ± 3.19^{bB}	69.25 ± 1.49^{cB}	<0.001
6w	68.5 ± 1.33^{aC}	77.25 ± 2.42^{bC}	80.5 ± 2.5^{bB}	0.009
8w	69.75 ± 1.17^{aC}	111.75 ± 6.03^{bD}	131.5 ± 3.5^{cC}	<0.001
P-Value	<0.001	<0.001	<0.001	

Data expressed as Mean ± stander error (N= 4 animals)
Different small letters among material groups in rows mean there is significant difference at $p\leq0.05$
Different capital letters among period in columns groups mean there is significant difference at $p\leq0.05$

TABLE 2. The means of the Osteoblast numbers in the all groups and periods

	Osteoblast No./40x field Mean±SE			
	Control	**Granules**	**Putty**	***P*-Value**
2w	24.2 ± 1.28^{aA}	41.25 ± 1.88^{bA}	69.25 ± 1.49^{cA}	<0.001
4w	44.6 ± 1.63^{aB}	74.2 ± 2.50^{bB}	80 ± 1.30^{bB}	<0.001
6w	73.2 ± 2.68^{aC}	47.25 ± 1.45^{bAC}	74.4 ± 2.20^{aC}	<0.001
8w	61.5 ± 2.66^{aD}	42.4 ± 2.06^{bAD}	35.75 ± 1.71^{bD}	<0.001
***P*-Value**	<0.001	<0.001	<0.001	

Data expressed as Mean ± stander error (N= 4 animals)
Different small letters among material groups in rows mean there is significant difference at $p\leq0.05$
Different capital letters among period in columns groups mean there is significant difference at $p\leq0.05$

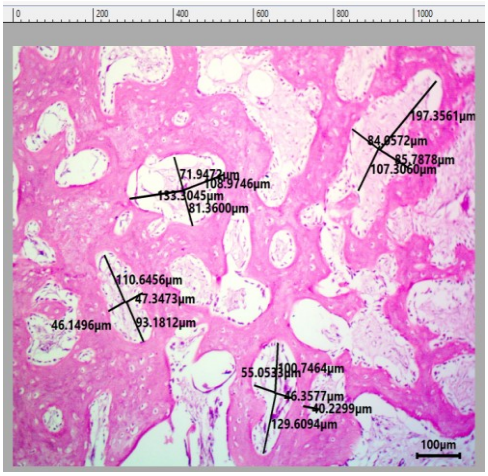

Fig. 2. microscope image of a sheep tissue with (bone marrow space) micromorphometric measurements.

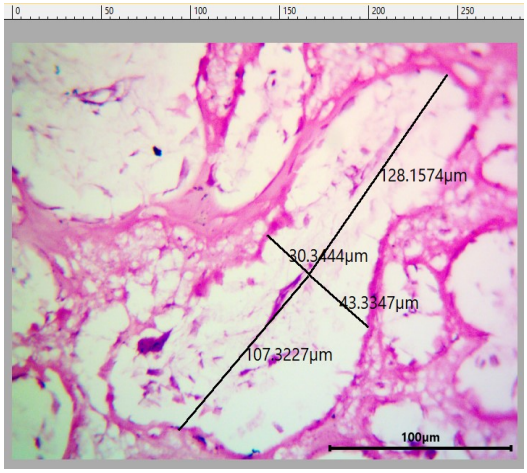

Fig. 3. microscope image of a sheep tissue with (bone marrow space) micromorphometric measurements.

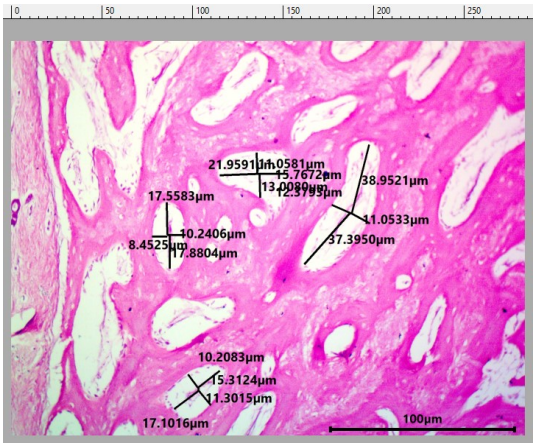

Fig. 4. Microscope image of a sheep tissue (bone marrow space) with micromorphometric measurements.

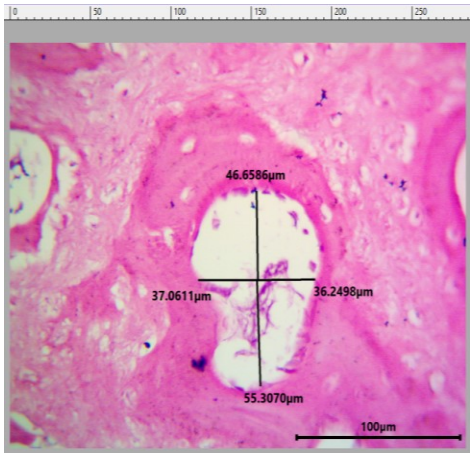

Fig. 5. Microscope image of a sheep tissue (bone marrow space) with icromorphometric measurements. Micromorphometric measurements: figures 2 to 5 showing a sheep tissue (bone marrow space) with micromorphometric measurements**.**

Immunohistochemistry study

In this study, histological sections of the sheep tibial bone were examined for Ki67 expression through immunohistochemical analysis in various experimental groups. The negative control group exhibited very weak Ki67 expression at 2 and 4 weeks, with a score of +1, as observed at both 100X and 400X magnifications, while at 6 and 8 weeks, Ki67 expression in this group increased to a moderate level, with a score of ++2, at both magnifications. In contrast, the granules group displayed a consistently moderate Ki67 expression at 2, 4, 6, and 8 weeks, with a score of ++2 at 100X magnification. Conversely, the putty group demonstrated intense Ki67 expression with a score of +++3 at 4 weeks and 8 weeks, both at 100X and 400X magnifications, while showing a moderate expression at 2 and 6 weeks. These findings indicate dynamic changes in cell proliferation within the sheep tibial bone under different experimental conditions and time points, as illustrated in the provided figures.

Each group had 4 specimens scored at periods (2 , 4, 6 and 8 weeks). The scores represent: 0 (- negative expression), 1 (+ weak positive expression), 2 (++ moderate positive expression), and 3 (+++ intense positive expression.

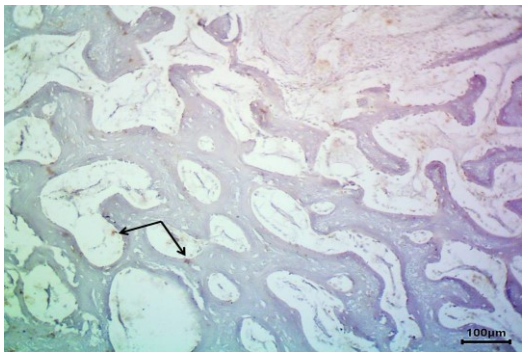

Fig. 6. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the negative control (2 weeks) group showing very weak expression (score +1); hematoxylin; 100X.

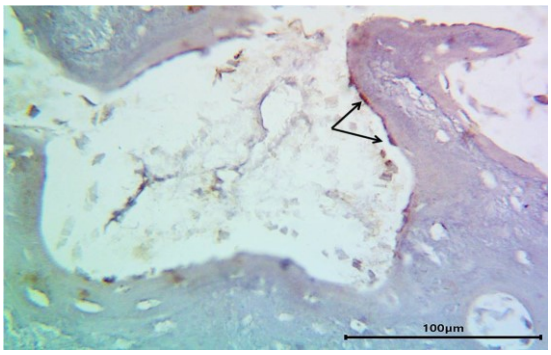

Fig. 7. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the negative control (2 weeks) group showing very weak expression (score +1); hematoxylin; 400X.

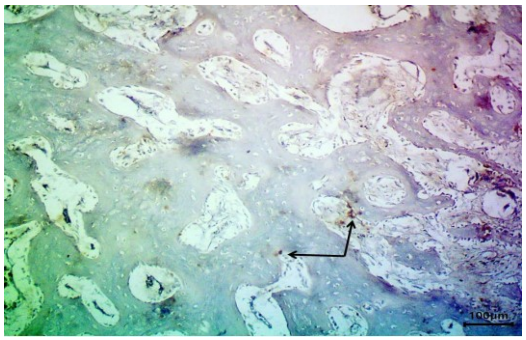

Fig. 8. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the negative control (2 weeks) group showing very weak expression (score +1); hematoxylin; 400X.

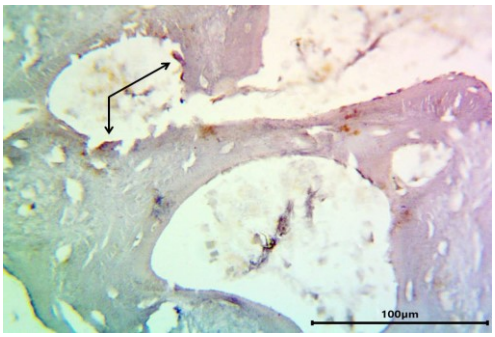

Fig. 9. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the negative control (4 weeks) group showing weak expression (score +1); hematoxylin; 400X.

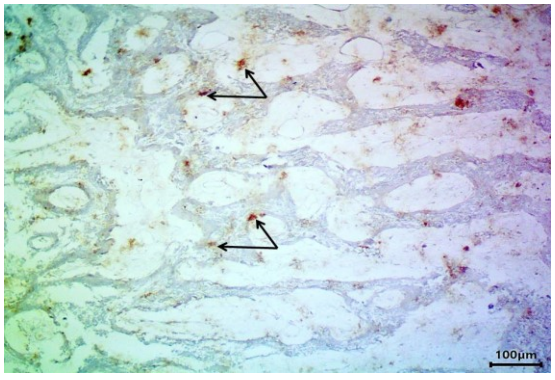

Fig. 10. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the negative control (6 weeks) group showing moderate expression (score ++2); hematoxylin; 100X.

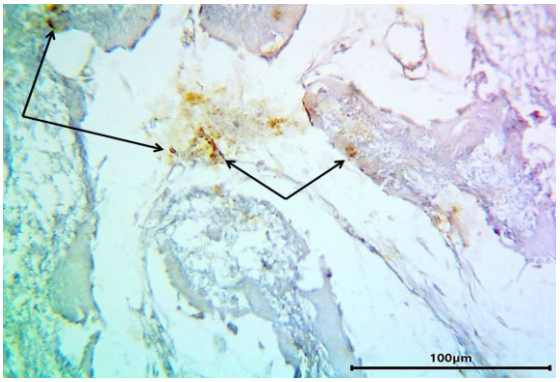

Fig. 11. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the negative control (6 weeks) group showing moderate expression (score ++2); hematoxylin; 400X.

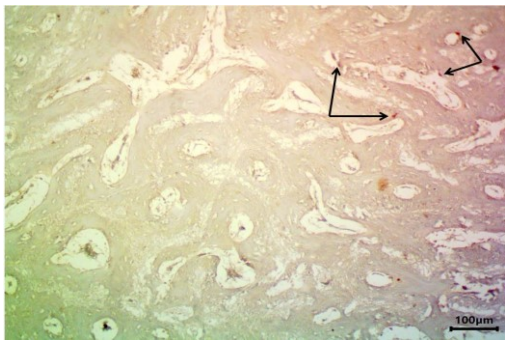

Fig. 12. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the negative control (8 weeks) group showing moderate expression (score ++2); hematoxylin; 100X.

Fig. 13. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the negative control (8 weeks) group showing moderate expression (score ++2); hematoxylin; 400X.

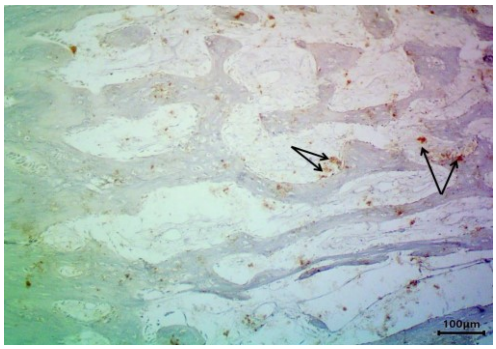

Fig. 14. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the powder (2 weeks) group showing moderate expression (score ++2); hematoxylin; 100X.

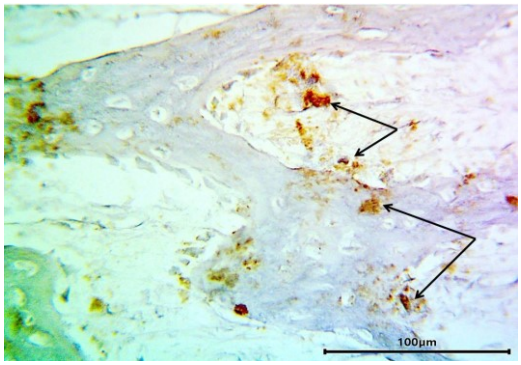

Fig. 15. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the powder (2 weeks) group showing moderate expression (score ++2); hematoxylin; 100X.

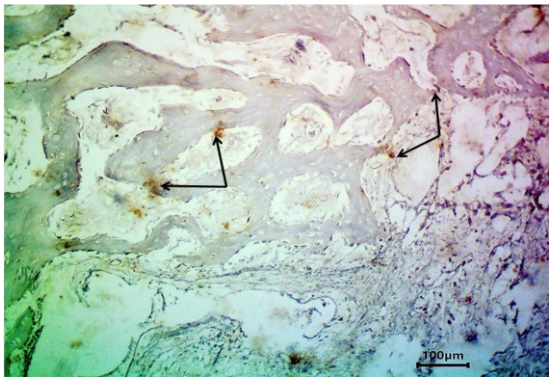

Fig. 16. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the powder (4 weeks) group showing moderate expression (score ++2); hematoxylin; 100X.

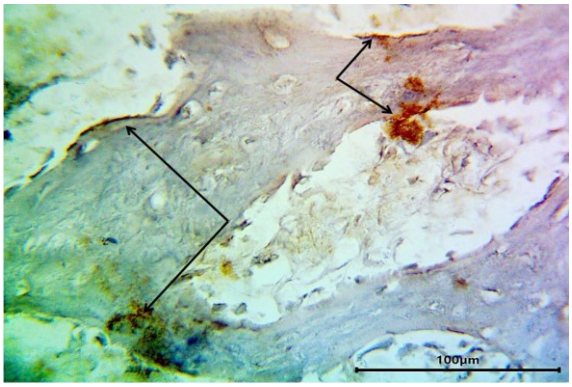

Fig. 17. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the powder (4 weeks) group showing moderate expression (score ++2); hematoxylin; 400X.

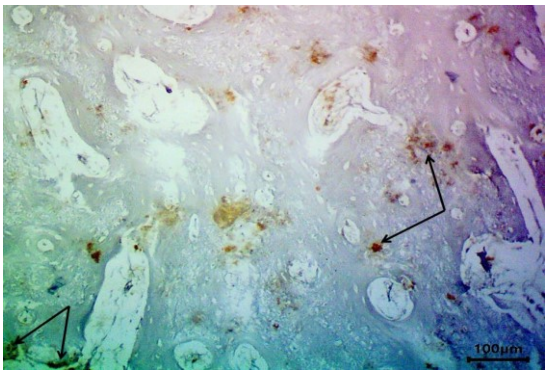

Fig. 18. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the powder (6 weeks) group showing moderate expression (score ++2); hematoxylin; 100X.

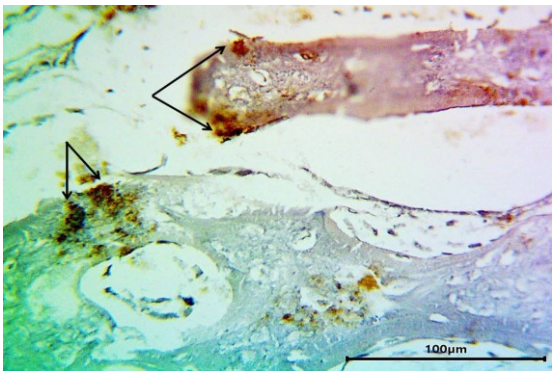

Fig. 19. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the powder (6 weeks) group showing moderate expression (score ++2); hematoxylin; 400X.

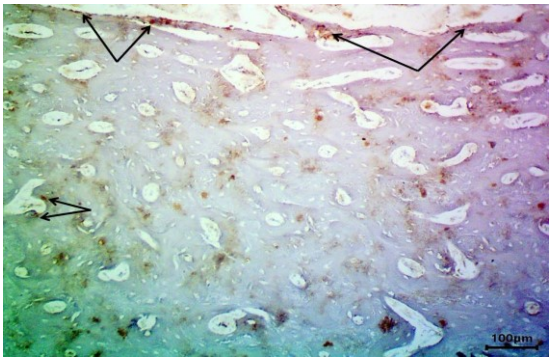

Fig. 20. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the powder (8 weeks) group showing intense expression (score +++3); hematoxylin; 100X.

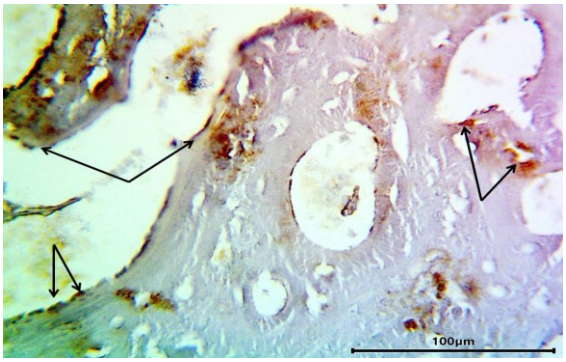

Fig. 21. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the powder (8 weeks) group showing intense expression (score +++3); hematoxylin; 400X.

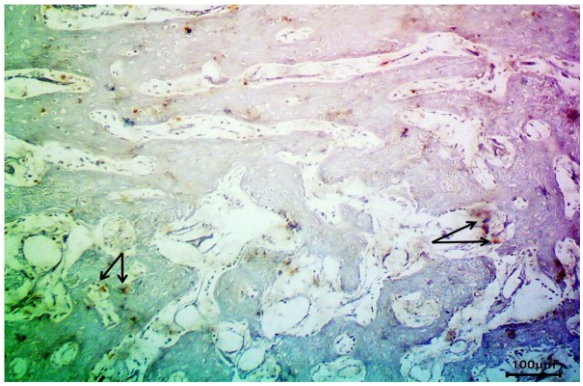

Fig. 22. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the putty (2 weeks) group showing moderate expression (score ++2); hematoxylin; 100X.

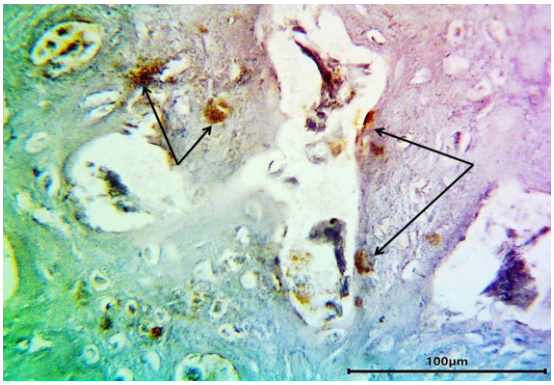

Fig. 23. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the putty (2 weeks) group showing moderate expression (score ++2); hematoxylin; 400X.

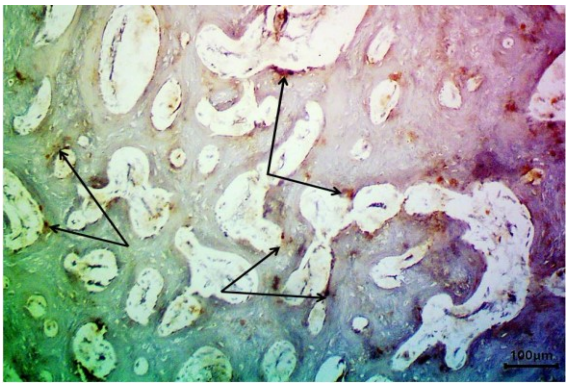

Fig. 24. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the putty (4 weeks) group showing intense expression (score +++3); hematoxylin; 100X.

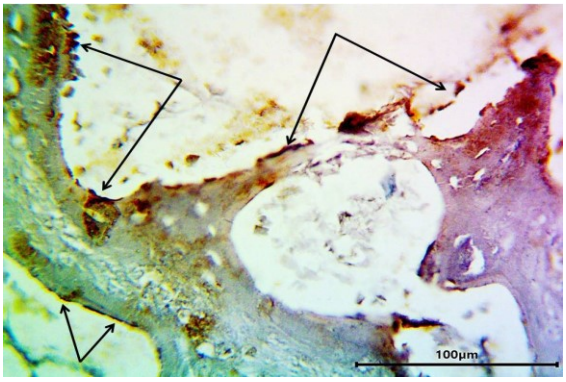

Fig. 25. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the putty (4 weeks) group showing intense expression (score +++3); hematoxylin; 400X.

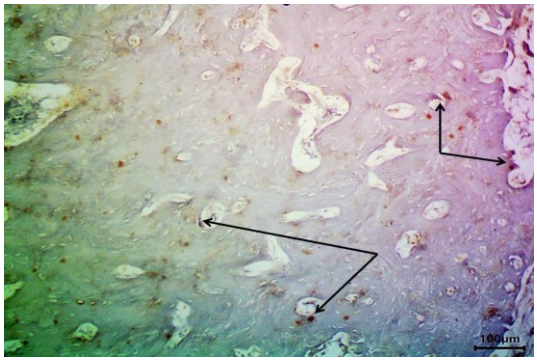

Fig. 26. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the putty (6 weeks) group showing moderate expression (score ++2); hematoxylin; 100X.

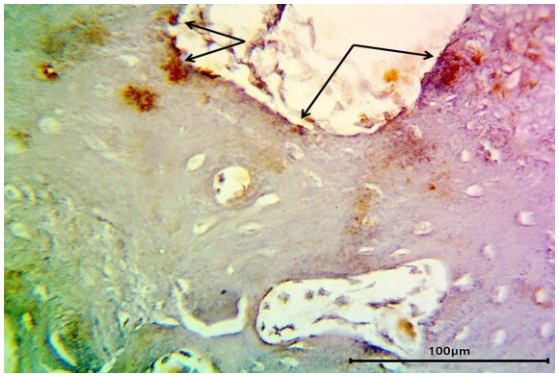

Fig. 27. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the putty (6 weeks) group showing moderate expression (score ++2); hematoxylin; 400X.

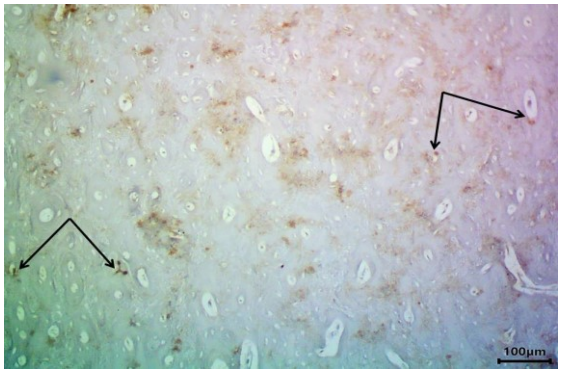

Fig. 28. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the putty (8 weeks) group showing moderate expression (score ++2); hematoxylin; 100X.

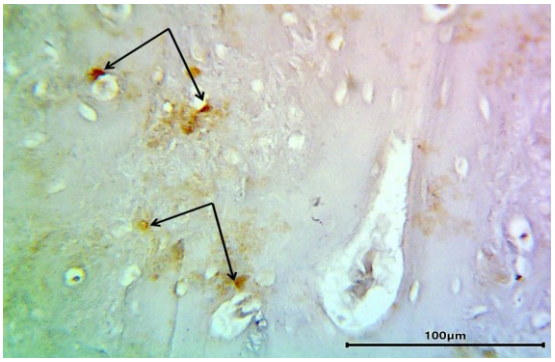

Fig. 29. Histological section of the sheep tibial bone of immunohistochemical expression for Ki67 of the putty (8 weeks) group showing moderate expression (score ++2); hematoxylin; 100X.

Table 3 and Figure 29 show immunohistochemical expression scores for Ki67 in different groups and time periods. Lowercase letters indicate significant differences between material groups (Control, Granules, and Putty) at corresponding time periods. For 4-week and 6-week periods, p-values of 0.030 and 0.055, respectively, indicate significant differences in Ki67 expression.

The study showed that the granules group showed moderate Ki67 expression levels at 2 weeks, 4 weeks, 6 weeks, and 8 weeks, with a median score of 1.5, 2, 2, 2.5, and 3, respectively, with no significant difference compared to the control group.

Putty Group in 2 Weeks (2w) and 6 Weeks (6w) The median scores are 2, indicating a moderate level of Ki67 expression. There is no significant difference compared to the Control group at these time points. At 4 Weeks (4w) and 8 Weeks (8w): The median scores are 3, indicating intense Ki67 expression. a significant increase compared to the Control group at 4 and 8 weeks.

TABLE 3. The scores of the immunohistochemical expression for Ki67 in the all groups and periods Scores of Ki67 expression, Median and IQR(Inter-Quartile-Range)

	Control	Granules	Putty	*p*-Value
2w	1 (1)^{Aa}	1.5 (1)^{Ba}	2 (1.25)^{Aa}	0.557
4w	1 (1)^{Ab}	2 (1.25)^{ABab}	3 (2.25)^{Aa}	0.030
6w	1.5 (1)^{Aa}	2.5 (2)^{ABa}	2.5 (2)^{Aa}	0.055
8w	2 (1.25)^{Aa}	3 (2.25)^{Aa}	2 (2)^{Aa}	0.260
***p*-Value**	0.454	0.039	0.117	

Data expressed as Median of the scores (N= 4 animals)
Different small letters among material groups in rows mean there is significant difference at $p \leq 0.05$.
Different capital letters among period in columns groups mean there is significant difference at $p \leq 0.05$.

Statistical analysis

Computer package (Sigma Stat V12.0 / SYSTAT software) was used to conduct the histomorphometrical analysis. Data were presented as means ± SE (standard error) and were analyzed by One Way ANOVA test using Duncan's test with significant level set on $P < 0.05$.

Discussion

Sheep were selected in experience, because it is bones are histologically and biochemically similar to human bones, as well as their ease of handling and low cost [11, 12]. These features make sheep an excellent model for experiments on bones [13].

TCP granules is employed as a scaffold for bone cells to adhere to, as well as to link bone fractures, indicating that it promotes the formation of new bone tissue [14].

The study showed an increase in the number of osteocytes compared to the control after two weeks of treatment with TCP granules and putty, and this indicates the beginning of the formation of new bone tissue, as shown by a previous study [15].

TCP granules initially produces irritation and swelling because it increases inflammation, which attracts white blood cells and other elements that aid in healing to the damaged area. This granule is absorbed and replaced by new bone tissue as the healing process advances. The healing pace is determined by the porosity of the granules, since larger and more poisonous granules is absorbed slowly and is an excellent alternative for repairing major bone lesions that require lengthy healing periods. It is critical to understand that the material it is not a replacement for surgery, particularly in fractures where the bones must be straightened [16]. In general, β-TCP releases calcium and phosphate ions that the bone needs for repair and construction and to compensate for the deficiency in bone tissue [17]. This substance is also an effective alternative in treating some spinal conditions and dental implants [18].

After two weeks of treatment with putty β-TCP The result showed an increase in the number of osteocytes and Osteoblast compared to the control after two weeks of treatment with Putty TCP, and this indicates the beginning of the formation of new bone tissue, as shown by a previous study [15].

After 4 weeks of treatment with granules β-TCP The study revealed an increase in osteocytes and osteoblasts after 4 weeks of treatment with TCP granules, indicating the start of new bone tissue development. [19].

After 4 weeks of putty β-TCP treatment (Fig. 22) shows a small number of bone cells and blood capillaries, indicating a healing process. is very close to completion [20]. These results indicating the beginning of the creation of new bone tissue, as revealed by a previous study [21].

Treatment with β-TCP granules after six weeks appearing formation of new capillaries that are essential for providing nutrients and oxygen to the healing bone tissue. Their increased presence in Figure 10 suggests that the healing process is progressing well. The study discovered an increase in the number of osteocytes and osteoblasts after 6 weeks of continuous β-TCP granules treatment compared to the control, indicating the beginning of the creation of new bone tissue, as reported by a previous study [21].

After six weeks of treatment with β-TCP putty the results show there is progress in the healing process, as well as a regular bone circumferential area, and that the procedure is nearly complete. In Figure 24 demonstrates that the number of osteocytes is low, indicating that complete healing

is near. There is also putty material scattered throughout the bone marrow area.

When treatment with β-TCP granules (after eight weeks) there are an increase in the number of osteocytes and osteoblasts compared to the control, indicating the beginning of the creation of new bone tissue, as established by another previous study [22]. While after eight weeks of treatment with β-TCP putty, the results recorded an increasing in the number of osteocyte and a decrease in the number of osteoclasts when using the β-TCP putty. This is consistent with what was mentioned previously because the bone building process has been completed or is nearly complete.

The best choice of material (granules or putty) for a particular patient will depend on the specific circumstances of the case [23].

both materials have shown effectiveness in promoting bone healing, the β-TCP putty appears to offer some improved changes in the healing process [24].

The time of absorption of Beta-TCP differs between granules and putty forms, with the putty absorbing faster than the granules and taking 2-12 months to absorb, whereas the granules takes 6-24 months [25].

It was found that Granules-TCP and Putty-TCP caused an increase in the number of osteocyte cells in different periods of 2-6-4 weeks, with a difference in the 8 week period in putty-TCP, which showed a greater increase in the number of osteocyte cells, while the number of osteoclast cells was decreased in 8 week periods. Which may indicate that the healing process is approaching the end and there is no longer a need for more new osteoclast cells (26).The increase in osteocyte cells suggests that both the granules and putty-TCP have an effect on the interior microenvironment of the bones at all times [27, 28].

Ki67 antigen is a expressed nuclear protein in all proliferating cells and is a valuable method for quantitative estimation of cell growth by immunohistochemistry [29].

The immunohistochemistry study involving Ki67 expression in sheep tibial bone sections provides valuable insights into the cell proliferation dynamics under the influence of Granules β-TCP and Putty β-TCP at various time points [30].

The Granules group showed moderate Ki67 expression, indicating sustained cell proliferation, and a more stable response compared to the negative control group, suggesting potential positive effects.

The Putty group showed moderate Ki67 expression for 2-6 weeks, similar to the negative control group. At 4 and 8 weeks, it showed intense Ki67 expression, indicating a significant increase in cell proliferation. Both groups showed temporal changes.

The study shows that Granules β-TCP and Putty β-TCP influence cell proliferation in sheep tibial bone, with Granules showing a controlled impact and Putty stimulating cell proliferation, suggesting material composition and form play a role [30].

Conclusion

Both forms showed a good response to the healing of the bone defect, although. The putty form of β-TCP was better, had faster resorption, and less inflammation after 8 weeks of treatment compared to the granular form.

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Author`s contribution:

The second researcher proposed the topic, and both the first and second researchers participated in designing the experiment and implementing it. The first researched its practical form, and the second researcher participated with him in writing and analyzing the results.

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تأثير الشكل المعجوني والحبيبي لبيتا-تري فوسفات الكالسيوم على القياسات النسجية للخلايا العظمية وتعبير Ki 67 في عظم قصبة الأغنام

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الخلاصة

الخلفية العلمية : تعتبر المدة الزمنية لشفاء الكسور العظمية في الانسان الحيوانات امرا بالغا الاهمية لهذا تسعى الدراسات لايجاد مواد تساهم في شفاء اسرع ونتائج افضل.

الهدف: استهدفت هذه الدراسة مقارنة تأثير معجون وحبيبات ثلاثي فوسفات الكالسيوم على شفاء عيب العظام في الأغنام.

المواد و طرائق العمل: شملت الدراسة 4 أغنام ذكور. خضع كل حيوان لعملية جراحية لعظم الساق بإحداث ثلاث عيوب في الساق كان الأول لوضع مادة الحبيبات والثاني للسيطرة والثالث لوضع مادة العجينة، مع فترة مراقبة مدتها من 2-8 أسابيع. تم اجراء الموت الرحيم للحيوانات بعد نهاية التجربة.

النتائج: لوحظت اختلافات معنوية في عدد الخلايا العظمية بين مجاميع الحبيبات ومجموعة العجينة مقارنة مع السيطرة في الفترة الزمنية المختلفة، واظهر كلا من الشكلين وجود زيادة في عدد الخلايا العظمية مقارنة مع السيطرة في الفترات الزمنية الأربعة، تبين وجود الالتهاب في المراحل المبكرة من العلاج بالشكل الحبيبي مقارنة بالشكل العجينة، كما سجل انخفاض معنوي بعدد الخلايا البانية للعظم في مجموعة العجينة بعد 8 أسابيع من المعاملة، وتبين ان سرعة ارتشاف مادة العجينة كانت اسرع في الشكل العجيني منه في الحبيبات كما ان سرعة استجابة العظم للشفاء كانت اسرع في نفس المدة . عرضت مجموعة الحبيبات تعبير Ki67 معتدلًا باستمرار في 2 و4 و6 و8 أسابيع، بدرجة ++2، وعلى العكس من ذلك، أظهرت مجموعة المعجون تعبير Ki67 مكثفًا بدرجة +++3 في 4 أسابيع و8 أسابيع.

الاستنتاج: اظهر كلا من الشكلين استجابة جيدة لشفاء العيب العظمي رغم ان المعاملة بشكل العجينة β-TCP كانت افضل واسرع ارتشاف واقل التهاب بعد 8 أسابيع من المعاملة مقارنة مع الشكل الحبيبي.

الكلمات المفتاحية: قصبة الساق، الخلايا العظمية، بانيات العظم، الأغنام.