



The radiological study of using fabricated calcium hydroxide from quail eggshell and plasma-rich fibrin for reconstitution of a mandibular bone gap in dogs

A.G. Atiyah¹, L.M Alkattan² and A.M. Shareef³

¹Department of Surgery and obstetric, College of Veterinary Medicine, University of Tikrit, Tikrit, ²Department of Surgery and Theriogenology, College of Veterinary Medicine, University of Mosul, ³Department of Radiology, Collage of Medicine, Nineveh University, Mosul, Iraq

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Correspondence:

A.G. Atiyah
alighazivet@tu.edu.iq

Abstract

In this study, Calcium hydroxyl was fabricated from quail egg-shell and autogenous plasma-rich fibrin (PRF) to reconstitute the mandibular gap in dogs. In this study, 27 dogs of both sexes were used, enrolled in three groups, nine of each. A defect as a circular gap experimentally induced on the ventral surface of the lower mandible with a diameter of (14,0.5 mm). Clinical and Radiographical examinations were evaluated at (0,15,30 and 60 days post-surgery), and the XRD (X-Ray Diffractometer), Field Scanning Electron Microscopy (FESEM), and Energy Dispersive X-ray Spectrometer (EDS) analysis were performed. Clinically there was normal mastication and no award complications. Radiographically in 1st group treated with Ca(OH)₂, the healing near completed, and the opacification of the bone defect in the caudal body of the mandible, with a sclerosed margin representing maturing callus with complete trabecular bridging, whereas in 2nd group at same period representing good maturing callus with complete trabecular bridging, there is disappearance of gap and complete opacification. The XRD scanning of the quail eggshell proved the hexagonal crystalline shape of calcium hydroxide containing essential elements of natural bone calcium, oxygen, and Carbone. At the same time, FESEM demonstrated the characteristic hexagonal shape of the particles, allowing identifying them as calcium hydroxide in Ca(OH)₂ group with no porous in PRF. In conclusion, using fabricated calcium hydroxide quail egg shell and prepared autogenous PRF demonstrated an effective bioactive agent with superior biocompatible properties of PRF for reconstitution mandibular defect in dogs; there was increased radiographic density of defective bone.

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Introduction

The mandibular defect and fracture comprise the second most common facial bone fracture. Mandibular continuity defect is the loss of a portion of the bone in the lower jaw (1). Causes of mandibular bone defect can be acquired. Rare genetic causes, trauma, malignant and benign tumors, and chronic osteomyelitis can cause significant mastication impairment (2). Therefore, the healing process of the

mandibular bone is complex and is considered a challenge for orthopedic surgery. Furthermore, the reconstitution of mandibular defects remains a real challenge for the reconstructive surgeon from both a functional and an aesthetic point of view (3). Many factors affect the healing process and contribute to the delay of this process, such as mechanical stress during mastication, the complex anatomy of the jaw, limited availability of appropriate soft tissue, poor blood supply and presence of inflammatory periodontal

diseases, all these factors may delay the bone healing process of the mandible (4). Currently, with the rapid development of biological materials and the need for modern regenerative medicine, research on bone substitute biomaterials that have been applied to mandibular defects is becoming increasingly important, such as bioceramics (5) and calcium phosphate-based biomaterials (6). Also, using autologous bone grafting in orthopedic surgery as tibial bone (7) and maxillofacial surgery (8). Bone substitute biomaterials have been included as therapeutic strategies for bone regeneration, especially in critically damaged bones (9). The aim of using a bioactive material is to reinforce the bone tissue healing process in the defective area, bioactive materials enhance bone regeneration and have beneficial value in clinical applications, especially in the treatment of non-union fractures and for the reconstitution of bone defects these advantages in improving the biological characteristics due to the limitations of a single material in terms of biological, physical and chemical properties (10). The PRF is considered an autogenous biomaterial product with a higher leukocyte count and fibrin mesh act as a gelatinous scaffold to increase osteogenic cellular migration and proliferation, and as an antimicrobial effect in tissue regeneration (11). PRF's biological and clinical features make it widely used in the medical field for treating skin ulcers or necrosis, plastic and reconstructive surgery, and musculoskeletal lesions, especially in orthopedic surgery used in bone regeneration suffered from critical and non-critical defects (12,13). PRF has several applications in the veterinary field, especially in oral and maxillofacial surgery, such as gingival recession, intra-bone defects, alveolar filling after extraction, and sinus lifting. PFR is vital in increasing the bone deposition rate and improving bone regeneration quality. It has several growth factors (14,15). Another natural bone substitute is recycling quail eggshells for bone regeneration. This pre-prepared agent is used with starch, 64S bioactive glass, and aloe vera leaf to promote osteoconductive and osteoinductive characteristics through this combination as a scaffold for bone regeneration (16). The quail eggshell (*Coturnix coturnix*) contains a higher amount of calcium than the eggshell of a hen and duck eggshell since it does not contain high levels of protein (17,18).

This trial uses fabricated calcium hydroxyl from quail eggshells and autogenous plasma-rich fibrin (PRF) from collected blood to reconstitute mandibular gaps in dogs.

Materials and methods

Ethical approve

All surgeries and animal care were performed according to Animal Care and Committee procedures and approved protocols at the University of Mosul, College of Veterinary Medicine No: UM.Vet.2022.050.

Animals and surgical preparation

Twenty-seven adult healthy stray dogs of both sexes, weight, and age (17 ± 0.8 Kg, and 12 ± 1.1 months, respectively) were included in this study. The experiment was divided into three equal groups, nine of each, control (CG), fabricated quail eggshell (FGEG), and autogenous (PRFG). Surgeries were performed under a protocol of general anesthesia ketamine of 15 mg/kg and Xylazine of 5 mg/kg of body IM, respectively (19,20).

The animals were restrained on lateral recumbence. The ventral surface of the lower mandible is prepared aseptically. Approximately 4 cm skin incision along the ventral part of the mandible in the submandibular region), the mandibular bone exposed, using a stainless-steel bone drill, circular bone defect as an experimentally induced with a diameter of 14 mm and 0.5 mm depth under continuous irrigation with the saline solution without perforation of the oral mucosa (Figure 1). The first group lifted without any treatment; the first treatment group defect performed as in the control group but filled with prepared CaOH_2 fabricated from quail eggshell, which was considered a bone substitute (Figure 2). The synthesis of CaOH_2 from the shell of the quail was performed by hydrothermal method.

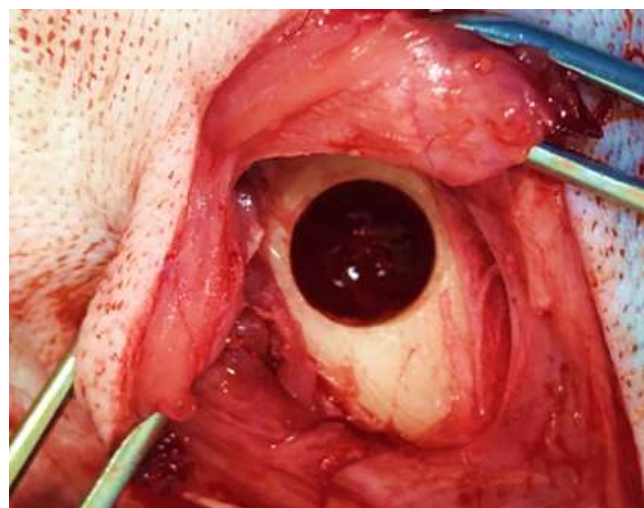


Figure 1: Experimental mandible defect as the gap.



Figure 2: prepared Ca(OH)_2 from quail eggshell, considered bone substitute filling material.

PRF preparation

The PRF preparation was according to the PRF method in the dog (21). 10 mm blood from the dog's jugular vein was harvested aseptically and centrifuged at 3000 rpm for 10 min. After centrifugation, the middle layer contained PRF; this gelatinous agent was collected and applied directly in the gap (Figure 3).



Figure 3: Prepared PRF considered as bone substitute filling material.

XRD analysis

The XRD analyses were performed to analyze the crystal phase of the quail eggshell Ca(OH)_2 , the XRD data were recorded within the two thetas (2θ) range from zero $^\circ$ to 80 $^\circ$, and intensity counts range from zero to 4900 $^\circ$, used (Crystalloflex-diffractometer, D- 500, Siemens. Germany), with an electrical voltage of zero kV to 900 kV (22,23).

FESEM test

The surface morphology and crystal size with porosity of the quail eggshell Ca(OH)_2 powder particles and the PRF sample were detected by using the (FESEM) model (inspect f 50, fei, Holland). Before electron microscopy, the samples were subjected to a gold covering to give the necessary conductivity.

EDS test

The element compositions of the quail eggshell Ca(OH)_2 powder sample with element mapping were obtained using an EDS system and through an acceleration voltage range from zero to 20 keV.

Results

The results of the radiological investigation in zero time of the induced mandibular defect as a gap in the lateral view of the ventral indicated a well-defined punched rounded radiolucent bone defect in the caudal body of the right mandible; however, it looks clear, no trabecular bridging nor definite significant callus formation (Figure 4). Whereas at 30 days post-operative, it indicated a partial sclerosis margin. A relatively increased density in the defect's center represents a developing callus with partial trabecular bridging. (Figure 5), Finally, at 60 days, a relative increase

in density is seen in the defect that represents the mature callus with almost complete trabecular bridging (Figure 6).



Figure 4: The lateral view of the ventral mandible in the control group at zero time indicated well-defined punched rounded radiolucent bone defect.



Figure 5: The lateral view of the ventral mandible in the control group at 30 days indicated a partial sclerosis margin, and there is a relatively increased density seen in the center of the defect.

The results of radiological imaging in the treatment group (FGEG), At 30 days after the operation, a round radiolucent bone defect in the caudal body of the Right mandible was indicated, with a nearly complete sclerosed margin and a relatively increased density was observed throughout the

defect representing developing callus with a near complete trabecular bridging (Figure 7).



Figure 6: The lateral view of the ventral mandible in the control group at 60 days represented a relative increase in density seen in the defect that represents the mature callus.



Figure 7: The lateral view of the ventral mandible in the treatment group FGEG at 30 days indicated a nearly complete sclerosed margin and a relatively increased density.

Whereas at 60 days post-surgery, the defective site near complete opacification of the bone defect in the caudal body of the right mandible, with a sclerosed margin representing maturing callus with complete trabecular bridging (Figure

8). In the PRF-treated group at 30 days, there was a sclerosed margin and opacification seen throughout the defect that represents the early maturing callus with near-complete trabecular bridging (Figure 9), while 60 days post-surgery sclerosis of the area representing good mature callus with complete trabecular bridging, there is disappearance (complete opacification) (Figure 10).

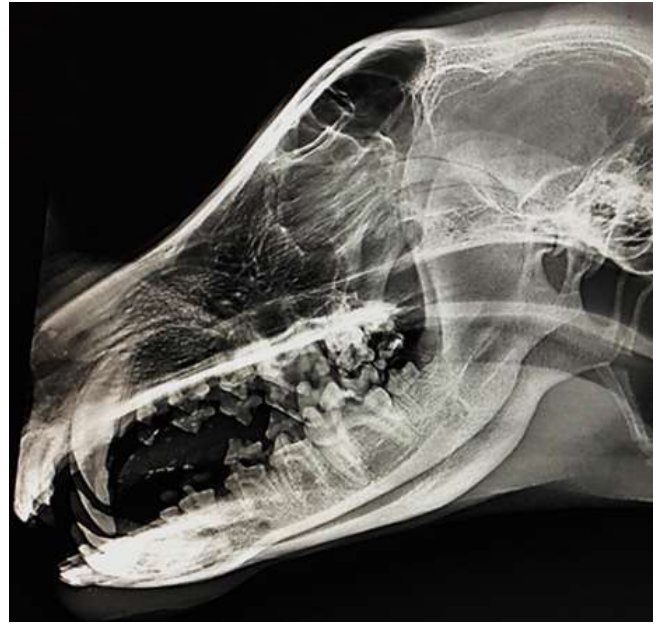


Figure 8: The lateral view of the ventral mandible in the treatment group FGEG at 60 days represents maturing callus with complete trabecular bridging.



Figure 9: The lateral view of the ventral mandible treatment group with PRF at 30 days represents the early maturing callus with near-complete trabecular bridging.



Figure 10: The lateral view of the ventral mandible treatment group with PRF at 60 days represents the early maturing callus with near-complete trabecular bridging.

XRD analysis

The outcomes of XRD patterns of the synthesized Ca(OH)_2 powder sample revealed that the typical intense peaks were detected with high crystallinity at 2θ (7.88° , 18.08° , 18.15° , 28.78° , 34.21° , 47.26° , 50.90° , 54.47° , 62.70°) respectively associated with the hexagonal crystalline shape of calcium hydroxide. All these peaks were matched with standard JCPDS card number (00-100-0045) using software Qualx version 2.25 (Figure 11).

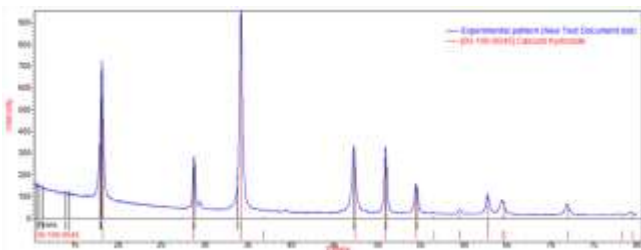


Figure 11: The XRD peaks patterns in blue color indicate the Quail eggshell Ca(OH)_2 sample, match with JCPDS reference card number: (00-100-0045) red in color.

FESEM

The FESEM images of the quail eggshell Ca(OH)_2 powder sample reveals that the surface morphology appears spongy, with particles appearing as polygons but with no uniform shape, and these particles are observed to be agglomerated. Some crystals grow as flower petals around the central point and sometimes appear in a hexagonal shape (Figure 12). The characteristic hexagonal shape of these particles is considered calcium hydroxide. The FESEM

image of the PRF sample shown in (Figure 13), indicated that the surface morphology of the PRF sample appears as a dense, smooth homogenous morphology with a swirl appearance without any porosity.

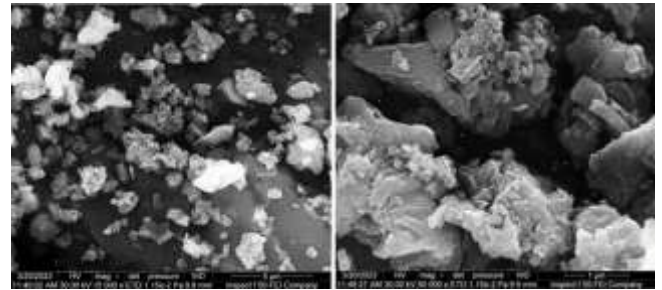


Figure 12: SEM image of the Quail eggshell Ca(OH)_2 obtained at the calcination temperature 1200°C , 15X and 30X.

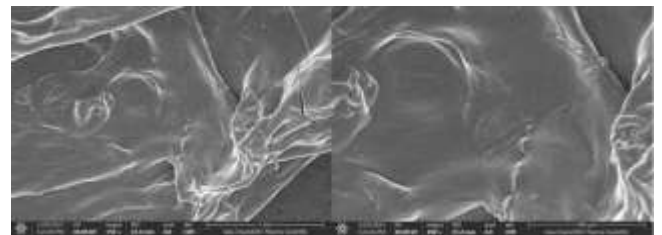


Figure 13: The FESEM images of the host PRF sample at magnification 150X and 300X.

EDS analysis

The EDS image and EDS elemental mapping of dried powder particles (Figures 14 and 15), respectively. Peak values at 3.691 and 0.532 keV confirms the presence of elements Ca and O, respectively. The EDS spectrum of Ca(OH)_2 shows the peak value at 45, 9, and 3 k, confirming the presence of elements Ca, O, and C, respectively. The calcium content was 44.5%, the oxygen content was 46.4%, and the carbon content was 8.5%. These values (weight %) are summarized in table 1. EDS elemental mapping showed that the particles contained calcium, carbon, and oxygen. The EDS mapping analyses were green, yellow, purple, and blue indicating the mapping of calcium, oxygen, carbon, and aluminum, respectively.

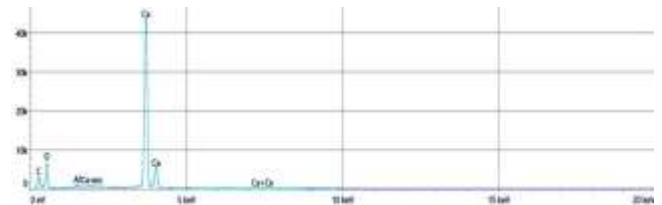


Figure 14: EDS spectrum peaks of quail eggshell Ca(OH)_2 powder sample.

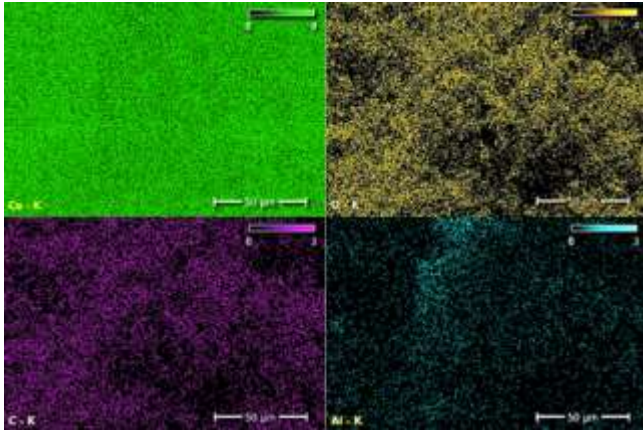


Figure 15: EDS elements mapping analysis of quail Ca(OH)_2 eggshell powder sample.

Table 1: Content values of quail eggshell Ca(OH)_2 powder

Elements	Weight %	Atomic %
Ca	44.5	23.4
O	46.4	61.2
C	8.5	15.0
Al	0.5	0.4

Discussion

The issues of orthopedic surgery still need to be investigated, including the optimal carriers for bioactive materials used alone or combined between different materials to stimulate synergistic effects. Mandibular defects account for nearly 59% of all facial defects, and their treatment can be complicated and complex with different anatomical features and postoperative infections due to infection with oral flora (23,24).

In this study, there was a promotion in the healing process and bone regeneration. This outcome was indicated clearly with the radiographic investigation at 60 days postoperatively in the PRF group, so it represented complete trabecular bridging and good maturing callus. The radiographic density of the treated defect increased significantly after PRF application; it provides a scaffold for growth factors and migration of osteogenic cells of new bone tissue formation and bone tissue regeneration. It improves musculoskeletal tissue healing (25-28).

In this study, the application of PRP indicated that it activates early bone regeneration, increasing the amount of new bone formation increases in trabecular density compared to the control group and the group these results disagree with Mohammadi *et al.* (29) who suggest that there are no effects of the PRF healing process of autologous bone tissue grafted mandibular gap in dogs. The PRF bioactive products contain a higher incidence of leukocyte cells and fibrin mesh. These act as a gelatinous scaffold to increase

osteogenic cellular migration and proliferation and act as an antimicrobial effect in tissue regeneration (30). In the treatment group Ca(OH)_2 , the formation of new bone and improving healing without infection due to The alkaline nature of Ca(OH)_2 . The ionic dissociation of Ca(OH)_2 releases hydroxyl ions that demonstrate its antimicrobial activity action. It is attributed to its ability to stimulate hard tissue formation and induce antimicrobial properties; it is a denatured protein and smashes cytoplasmic membranes and DNA (31,32). The scanning of fabricated quail eggshells indicated the presence of essential elements commonly formed in natural bone, like calcium, oxygen, and carbon, which originate from the quail eggshell, and these results agree with (33,34). The X-Ray diffraction of the powder sample indicated typical intense sharp peaks with high crystallinity of Ca(OH)_2 with less organic and carbonate material; these results were suggested by other workers (35). Thus, the synthesis of Ca(OH)_2 by hydrothermal method influenced in the control of the particle size, chemical composition and morphology of the fabricated bone substitutes biomaterials, and this method considers a low-cost process which can produce high crystallinity, easily controllable reaction, and environmentally acceptable method (36-40).

All these materials are used as filler materials to facilitate bone formation and promote bone healing, which are considered bioresorbable, do not undergo an antigen-antibody reaction, and act as a mineral reservoir that induces bone formation (32,34,39). Also, these materials act as biological scaffolds for improving osteogenic activity for regeneration of bone and play crucial role in tissue engineering (35).

Conclusion

The fabricated quail eggshell and prepared autogenous PRF demonstrated an effective bioactive agent with superior biocompatible properties of PRF for the reconstitution of mandibular defects in dogs. There was an increase in the radiographic density of defective bone.

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Conflict of interest

The manuscript has no conflict of interest.

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دراسة شعاعية لاستخدام هيدروكسيد الكالسيوم المصنوع من قشر بيض السمّان والبلازما الغني بالليفيين لإعادة تشكيل فجوة الفك السفلي في الكلاب

علي غازي عطية^١، ليث محمود القطان^٢ و احمد مذخر شريف^٣

^١ فرع الجراحة والتوليد، كلية الطب البيطري، جامعة تكريت، تكريت،
^٢ فرع الجراحة وعلم تناسل الحيوان، كلية الطب البيطري، جامعة
الموصل،^٣ فرع الأشعة، كلية الطب، جامعة نينوى، الموصل، العراق

الخلاصة

في هذا البحث، قمنا بتصنيع مسحوق هيدروكسيد الكالسيوم من قشرة بيض السمّان والليفيين الغني بالبلازما ذاتي المنشأ لإصلاح عيوب فجوة الفك السفلي في الكلاب. تم استخدام سبعة وعشرين كلبًا بالغًا يتمتع بصحة جيدة في هذه الدراسة موزعين في ثلاث مجموعات، ٩ في كل مجموعة. تم استخدام عيب عظمي دائري تجريبيًا على السطح البطني للفك السفلي بقطر ١٤ مم و ٠,٥ مم. تم تقييم الفحوصات السريرية والإشعاعية للفترات (١٥،٣٠،٦٠ يومًا) بعد العملية، تم تقييم المادة المصنعة باستخدام تحليل حيود الأشعة السينية، الفحص المجهر الإلكتروني للمسح الميداني، وتحليل مطياف الأشعة السينية المشتتة للطاقة. إجراء سريريًا كان هناك مضغ طبيعي، ولا توجد مضاعفات ظاهرة. إشعاعيًا المجموعة المعالجة بواسطة هيدروكسيد الكالسيوم، لوحظ اقتراب الشفاء من الاكتمال وظهور عتامة لعيب العظام في الجسم الذيلي للفك السفلي، مع وجود تصلب عظمي يمثل السفن الناضج مع اكتمال الجسور التريبية، بينما في مجموعة البلازما الغني بالليفيين في نفس الفترة لوحظ تكون السفن الناضج الجيد مع اكتمال الجسر التريبية، مع اختفاء فجوة العيب العظمي مع التعقيم الكامل لها. أثبت مسح الأشعة السينية لقشرة بيض السمّان الشكل البلوري السداسي لهيدروكسيد الكالسيوم مع احتوائه على عناصر أساسية من الكالسيوم والأكسجين والكربون والتي تعتبر من العناصر الموجودة طبيعيًا في العظام، بينما أظهر الفحص المجهر الإلكتروني للمسح الميداني شكلًا سداسيًا مميزًا للجزيئات، مما يسمح بتعريفها على أنها هيدروكسيد الكالسيوم في مجموعة هيدروكسيد الكالسيوم مع عدم وجود مسامية في عينة البلازما الغني بالليفيين. في الختام، فإن هيدروكسيد الكالسيوم المصنوع من قشرة بيض السمّان و البلازما الغني بالليفيين المحضر ذاتي المنشأ أثبتت أنها مواد حيوية جيدة لكن وجود خصائص التوافق الحيوي أفضل في عينة البلازما الغني بالليفيين يمكن استخدامها لإعادة تكوين عيب الفك السفلي في الكلاب، حيث انها تعمل على زيادة الكثافة الشعاعية للعيوب العظمية.