Research Article

BASRAH JOURNAL OF VETERINARY RESEARCH, 2023, 22(4):37-46
https://bjvr.uobasrah.edu.iq/

Study the Effect of Sodium Hyaluronate and Autologous Platelet Rich Fibrin on Symphysis Fracture Healing in Cat

Ibrahim Abdulzahraa Abbas[1], Luay Ahmed Naeem[1], Hassan M. Al-Tameemi[1].

1-Department of Veterinary Surgery and Obstetric, College of Veterinary Medicine, University of Basrah /Iraq.

Corresponding Author Email Address: ibrahamalafia@gmail.com

DOI: 10.23975/bjvetr.2023.181827

Received: 11 November 2023 Accepted: 11 December 2023.

Abstract

This study investigated the impact of sodium hyaluronate and platelet-rich fibrin on the healing process of symphyseal fractures in cats. To conduct this study, 30 adult male cats were utilized and divided into three groups (10 cats each). The groups consisted of a control group (no treatment), a sodium hyaluronate group (treated with sodium hyaluronate gel at the fracture site), and a sodium hyaluronate combined with platelet-rich fibrin group (treated with a mixture of sodium hyaluronate gel and platelet-rich fibrin at the fracture site). The symphysis fractures were induced via a surgical scalpel, and the fractures were then fixed using a cerclage wire. The cats were observed clinically on the first, second, third, and seventh days following the surgery, and Histopathological assessments were conducted 42 days after the surgery. The study showed improvement in the disappearance of swelling in the fracture area among the treated groups. Interestingly, cats were observed to regain the use of their jaws just three days post-surgery, which was not seen in the control group. Histological findings indicate complete fracture healing in the Sodium Hyaluronate-Platelet Rich Fibrin (SH&PRF) group, with active bone remodeling, cartilaginous callus formation, and peripheral fibrous reaction without inflammation. The SH&PRF group showed more active chondrocytes and endochondral ossification than the SH and control groups, suggesting better bone repair results. The study suggests that Sodium Hyaluronate and platelet-rich fibrin composite effectively promote and accelerate wound healing.

Keywords: Cat, Mandibular Symphysis Fracture, Sodium Hyaluronate, platelet.

Introduction

The mandible of the cat is characterized by its smaller size, and its manipulation implies using different repair procedures and repairing ways based on the size of the object to preserve its biomechanical functionality. In general practice, feline head trauma injuries are commonplace and often lead to mandibular fractures. The literature describes various methods of invasive fracture repair for feline mandibular fractures, such as mini-plates and screws, intramedullary or interfragmentary pins, external skeletal fixators, cerclage wires, and interfragmentary wires. However, some of these techniques have notable limitations when considering the mandibular fracture repair principles (1,2). The field of bone tissue engineering is a sophisticated interplay between cells, biomaterials, and signaling molecules, commonly referred to as a tissue engineering triad. Nonetheless, the latest advancements in regenerative medicine are centered on leveraging the body's innate ability to regenerate through engineered biomaterials, known as in-situ tissue engineering (3). Hyaluronic acid, a biomaterial known as hyaluronan with its conjugate base hyaluronate, is a non-sulfated glycosaminoglycan widely distributed throughout the body. Research has shown that treatment with hyaluronic acid can significantly enhance bone formation and stimulate the rate of wound healing, making it an effective therapeutic option in various medical applications. Hyaluronic acid is therefore a capable biomaterial and a potentially useful instrument for bone repair (4). Platelet-rich fibrin represents a more convenient and pragmatic alternative to traditional platelet-rich plasma. Platelet-rich fibrin is an autologous platelet concentrate that offers several advantages over traditionally prepared platelet-rich plasma. is derived from a patient's blood and prepared without any biochemical agents, making it easier to prepare and eliminating the risk of contamination from external sources. PRF is a more user-friendly and practical option than traditional PRP (5,6). An exciting era in tissue repair and regeneration has begun with the introduction of platelet-rich fibrin (PRF) as an autologous biomaterial in dental implantology, periodontology, oral surgery, and regenerative endodontics. The current study aimed to evaluate feline symphyseal fracture healing using sodium hyaluronate and autologous platelet-rich fibrin.

Materials and Methods

For the present study, thirty adult male cats were used and individually housed in cages. They were provided with free food and purified water for ten days before surgery to acclimatize them. The animals were divided into three groups, each consisting of (N= 10) cats. These groups were G1 control group, G2 Sodium Hyaluronate (SH) group, and G3 Sodium Hyaluronate and Platelet-Rich Fibrin (SH&PRF) group. To prepare autogenic Advance platelet-rich fibrin-plus (A-PRF+), a 5 ml blood sample is taken from a cephalic vein and promptly centrifuged at a speed of 1300 rpm for 8 min, as described previously (7). the platelets in the blood sample were activated upon touching the tube walls within a few minutes, triggering the coagulation cascades. Fibrinogen is initially accumulated in the top portion of the tube before being converted into fibrin by circulating thrombin. The middle of the tube contains the cellular layer at the top and red corpuscles at the bottom. Figure (1).

Surgical operation

The surgical site (the mandibular symphysis) was prepared by clipped and shaved, then washed thoroughly with water, followed by a 2–3-minute surgical scrub of the area using antisepsis (ethanol alcohol 70%) applied on the whole clipped area. Finally, the incision site was applied with 2.5 %tincture iodine. general anesthesia was accomplished by administering intramuscular injections of 2% xylazine hydrochloride at a dosage of 0.15 ml/kg of body weight and 10% ketamine hydrochloride at a dosage of 15 mg/kg of body weight. A surgical incision of 1-2 cm in length is made inside the mouth, starting from the midline between the incisor teeth and extending towards the lingual frenulum in the area of the mandibular symphysis using a surgical scalpel. A surgical scalpel was created a symphysis fracture in the lower jaw, resulting in an experimental symphysis fracture. The symphyseal fracture in all groups is fixed using a cerclage wire gauge 20 (0.8 mm) (Figure: 2(D) (8) (10). The fixed fractures of G1 were left without adding any materials to the fracture site. In the G2 group, sodium hyaluronate was applied locally to the fracture site. In the G3 group, a composite of sodium hyaluronate and platelet-rich fibrin was applied locally to the fracture site (Figure 2: B and C). Then, the incision site was closed routinely and the animals were monitored clinically and histopathologically the cats were observed clinically on the first, second, third, and seventh days following the surgery, and histopathological assessments were conducted 42 days after the surgery

Postoperative care: the animals) All the studied groups including the G1 group)were housed in a clean, dry area and given food, water, and antibiotics (ceftiofur) at a dose of 1 milliliter per 15 kilogram for five days. They were also given antipyretics, and the cerclage wire was removed 42 days after the healing had occurred.

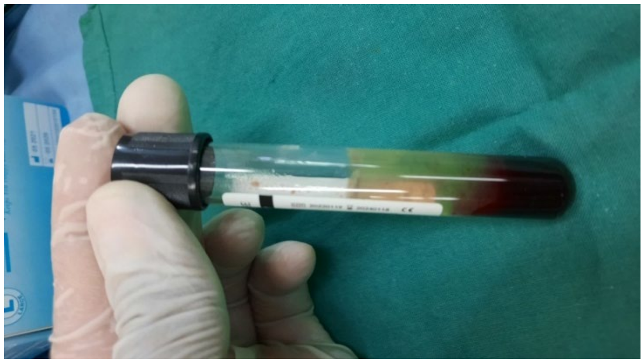

Figure 1: Advance Platelet-rich fibrin plus (A-PRF+)

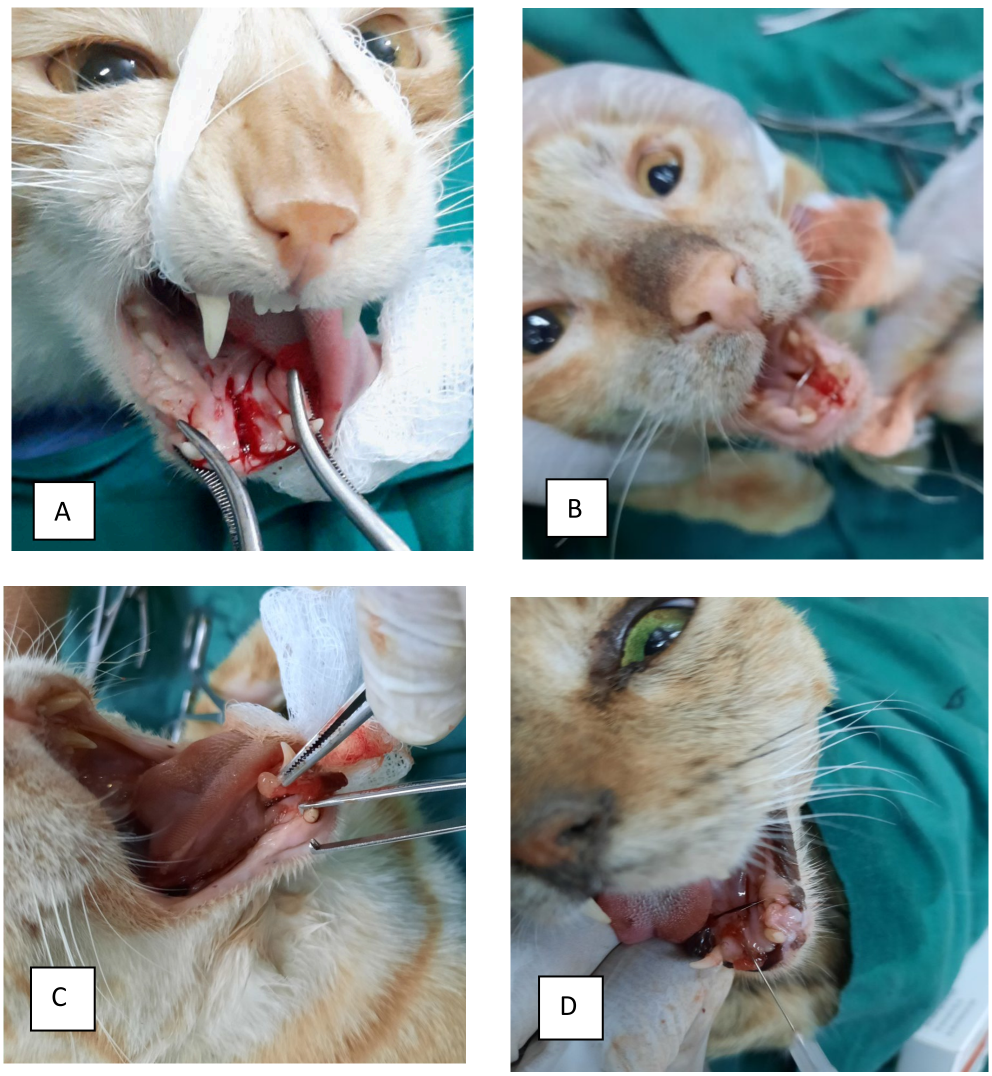

Figure 2-A: Intraorally incision from midline of canine teeth caudally to the lingual frenulum B: Fixation of symphyseal fracture. C: PRF treated. D : Hyaluronic acid injection.

Results

After the operation, there was pain in all groups of animals due to the fracture, and this pain decreases over the days. As for other signs, such as swelling, redness, loss of function, and Mobility of the fractured site, these signs are monitored during the first week after the beginning of the study. The swelling parameter was mild near the fixation edges in the control group figure(3-A) and absent in both treated groups figure(3-B&C) on the first day and clearly appearance on the second day of operation, the swelling was severe in the control group figure(4A) and moderate in both treated groups figure(4B&C); mild swelling was shown in all animal groups on the third day of operation; and on the seventh day of operation, the swelling was absent in all groups figure(5A,B and C).Severe redness in the gingiva was shown on the first day of operation figure(3A,Band C). In contrast, the redness was moderate, mild, and absent on the second, third, and seventh days of operation, respectively, in all animal groups figure(5A,B and C).Furthermore, loss of function was shown in all animal groups to be severe on the first day (figure4-1A,B and C), mild on the next two days (figure4-2A,B and C), and absent on the seventh day of operation (figure4-3A,B,and C).Mobility of the fractured site was the last parameter among clinical findings that showed mild mobility on the first and second days, and the mobility was absent on the third and seventh days of operation in all groups.

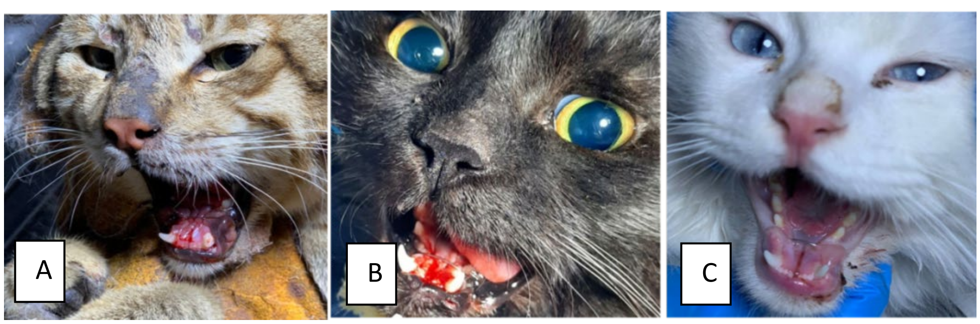

Figure- 3. first day A- (control group) , B- (SH group) C- (SH&PRF group)

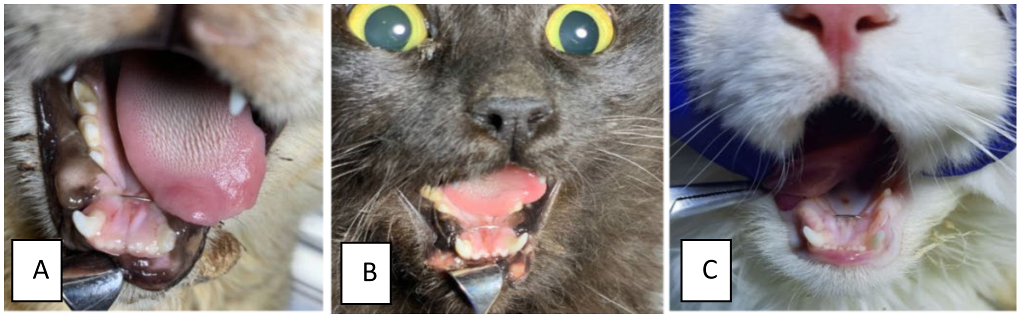

Figure -4 second day. A- (control group) B-(SH group) C-(SH&PRF group)

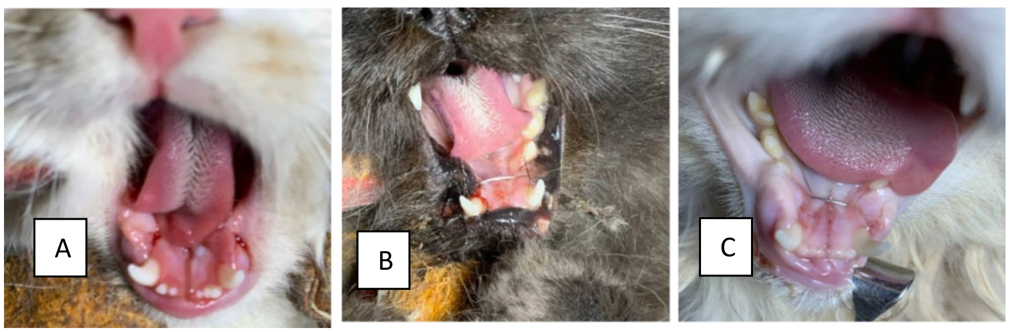

Figure 5: third and seventh day A-(control group) B-(SH group) C-(SH&PRF group)

The histopathological findings of each group provided valuable insights into the different stages and processes involved in bone repair. The control group exhibited immature cancellous bone, abundant collagen fibers, hypertrophic zone, and inactive bone marrow. Congested blood vessels were also observed (Figure 6). On the other hand, Group SH demonstrated cartilage formation and the presence of newly formed bone and fibrous tissue, along with newly formed active bone marrow and osteoblasts (Figure 7). Group SH&PRF exhibited a cartilaginous callus, active chondrocytes, endochondral ossification, active bone remodeling, and a peripheral fibrous reaction without inflammation. The periphery of the callus exhibited a novel osseous tissue formation, replacing the former cartilaginous elements. The bone tissue near the callus featured expansive medullary spaces, profuse fibroblasts, vascularity, and peripheral fibrous reactions within the cancellous matrix (Figure 8). These findings indicate variations in the stages and characteristics of bone repair in different experimental groups. Based on the information, Group SH & PRF has better bone repair results than the other groups.

.

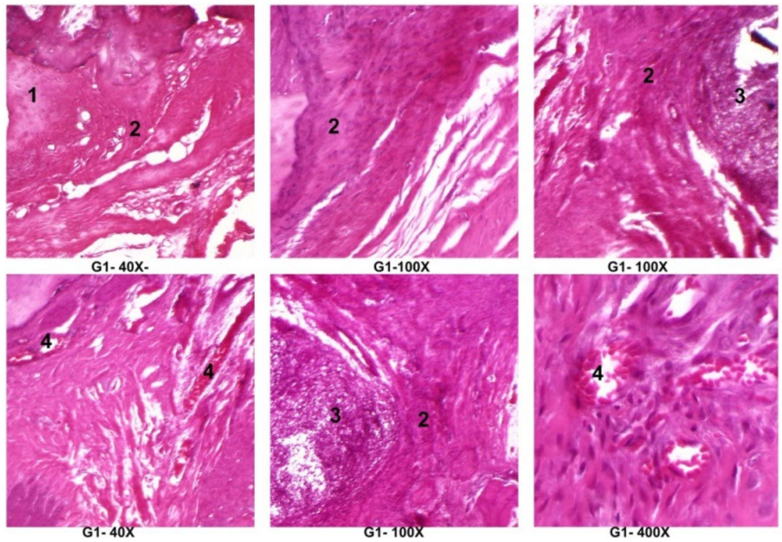

Figure 6: group control. Section of mandibular bone fracture after forty-two shows formation of an immature cancellous bone, fibrillar, shows abundant randomly distributed collagenous fibers (1), hypertrophic zone (2), mish of newly formed inactive bone marrow (3), congested blood vessels (4)

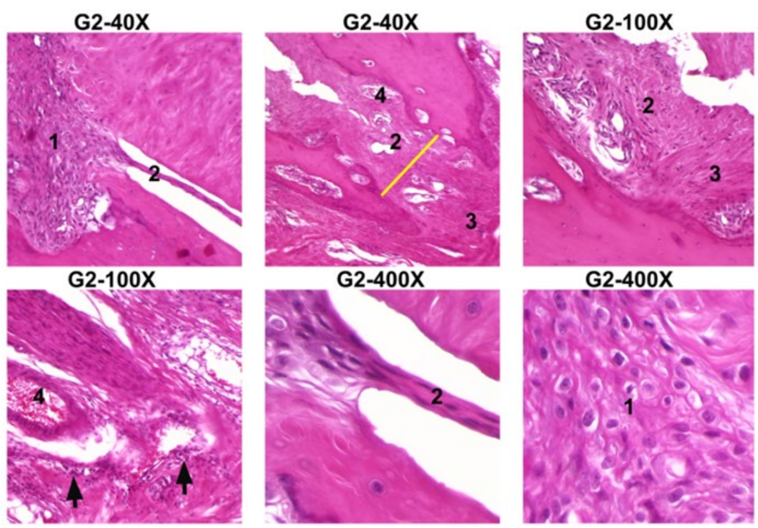

Figure 7: SH group. Section of mandibular bone after forty-two shows formation of cartilage (1), newly formed bone (2), newly formed fibrous tissue (3), newly formed active bone marrow (4), and newly formed osteoblasts (black arrows).

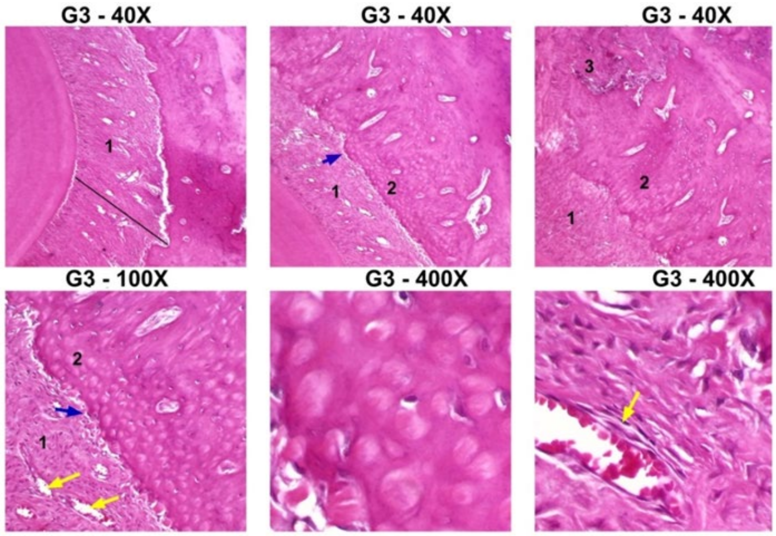

Figure 8: after forty-two SH&PRF group. Bone repair of mandible shows cartilaginous callus on the osteotomy line (2), active chondrocytes were evident, the area peripheral to the callus shows new bone formation whose cartilaginous components have been replaced by bone tissue employing endochondral ossification (1), Cancellous bone tissue next to the callus showing wide medullary spaces with abundant fibroblasts and blood vessels (yellow arrows), Peripheral fibrous reaction (3), without inflammatory elements were observed.

Discussion

Clinical observations from the present study revealed clear clinical signs, such as swelling, redness, loss of function, and impaired mobility at the fracture site, which were observed in all groups. However, compared to the control group, these signs disappeared in the groups that received sodium hyaluronate (SH) and SH-PRF three days after the operation. Our clinical evaluations demonstrated that the application of sodium hyaluronate - PRF composite locally on the symphyseal fracture site resulted in the disappearance of swelling around the fracture site. Moreover, the animals returned to their normal mandibular function, as the inflammatory stage of healing was accelerated, and soft tissue levels and appearance were restored to normal. These clinical findings are consistent with the efficacy of the sodium hyaluronate - PRF composite in enhancing the healing process of mandibular fractures. The CD44 receptor on the surface of leukocytes and endothelium binds with the HA molecule (11). This binding results in a reduction in the migration of white blood cells to the site of inflammation.

Consequently, there was mitigation the swelling at the wound site(12). According to reports, sodium hyaluronate and platelet-rich fibrin are imperative for ultimately healing fractures due to the two crucial roles hyaluronic acid (HA) plays in the initial stages of wound healing (12). HA in fracture healing is vital as it facilitates the transportation of nutrients, helps form a provisional extracellular matrix, and acts as a protective barrier against infections. Therefore, incorporating sodium hyaluronate and platelet-rich fibrin in fracture healing procedures is highly recommended to efficiently and effectively manage fractures.

A cartilaginous callus in the initial stage of bone repair characterizes the fracture healing process. Creating a soft tissue bridge composed of cartilage between the broken ends of the bone leads to the formation of a cartilaginous callus, providing stability and serving as a blueprint for subsequent bone formation through endochondral ossification. The formation of a cartilaginous callus is consistent with the early stages of bone repair (13). Active chondrocytes within the cartilaginous callus indicate that the cartilage is in flux and change. These specialized cells play a vital role in creating and maintaining cartilage tissue, implying that the callus is undergoing the crucial process of cartilage formation. This is a prerequisite for bone tissue production (14,10).. In the vicinity of the cartilaginous callus, new bone tissue is forming, indicating the conversion of cartilage into bone tissue through endochondral ossification (19). During this process, the cartilaginous matrix is gradually replaced by bone tissue, forming a bony callus. This transformation allows for the restoration of the bone's structural integrity (15) and (16). The cancellous bone tissue next to the callus has large medullary spaces filled with blood vessels and fibroblasts - cells that aid in connective tissue formation. This observation suggests active bone remodeling and revascularization, essential processes in the healing of fractured bones. Fibroblasts and blood vessels indicate active cellular and vascular contributions to bone repair, as stated in the research conducted by (17). A peripheral fibrous reaction is observed, indicating the presence of fibrous connective tissue around the healing site. The absence of inflammatory elements is noteworthy and suggests that the healing process occurs without significant inflammation. noted, excessive inflammation can impair the healing process and lead to complications (18) and (19). Therefore, the absence of inflammation is a desirable outcome.

Conflict of interest: All authors declare that there is no conflict of interest.

References

1-Freeman, A., & Southerden, P. (2023). Mandibular fracture repair techniques in cats: a dentist's perspective. *Journal of Feline Medicine and Surgery, 25*(2), 1098612X231152521.

2-Woodbridge, N., & Owen, M. (2013). Feline mandibular fractures: a significant surgical challenge. *Journal of feline medicine and surgery, 15*(3), 211-218.

3-Mansour, A. M., Yahia, S., Elsayed, H. R. H., El-Attar, S. A., Grawish, M. E., El-Hawary, Y. M., & El-Sherbiny, I. M. (2022). Efficacy of biocompatible trilayers nanofibrous scaffold with/without allogeneic adipose-derived stem cells on class II furcation defects of dogs' model. *Clinical oral investigations*, 1-17.

4-Zhai, P., Peng, X., Li, B., Liu, Y., Sun, H., & Li, X. (2020). The application of hyaluronic acid in bone regeneration. *International journal of biological macromolecules, 151*, 1224-1239.

5-Raja, V. S., & Naidu, E. M. (2008). Platelet-rich fibrin: evolution of a second-generation platelet concentrates. *Indian Journal of Dental Research, 19*(1), 42

6-Sharma, N. K. (2018). Platelet-rich fibrin: Emerging biomaterial in regeneration. National *Journal of Maxillofacial Surgery, 9*(1), 1.

7-Pavlovic, V., Ciric, M., Jovanovic, V., Trandafilovic, M., & Stojanovic, P. (2021). Platelet-rich fibrin: Basics of biological actions and protocol modifications. *Open Medicine, 16*(1), 446-454.

8-Atallah, F. A., Silva, R. S., Oliveira, A. L. D. A., & Souza, H. J. M. (2016). Subcolectomy and symphyseal distraction-osteotomy using a spacer of spirally fashioned orthopedic wire: a treatment option for cats with pelvic canal stenosis, megacolon and obstipation. *Ciência Rural, 46*, 1472-1478.

9-Muhammad, M. J., & Naeem, R. M. (2022). Effects of platelet-rich fibrin on skin pedicle graft healing: Clinical and biochemical study. International *Journal of Health Sciences, 6*(S5), 9253–9264

10-Scott, H., Marti, J., & Witte, P. (2022). Introduction to feline orthopaedic surgery. In Feline *Orthopaedics* (pp. 1-12). CRC Press.

11-Dovedytis, M., Liu, Z. J., & Bartlett, S. (2020). Hyaluronic acid and its biomedical applications: A review. E*ngineered Regeneration, 1*(November), 102–113

12-Akyildiz, Servet et al. 2018. "Acceleration of Fracture Healing in Experimental Model: Platelet-Rich Fibrin or Hyaluronic Acid?" *Journal of Craniofacial Surgery 29*(7): 1794–98.

13-Affshana MM, Priya J. 2015. Healing mechanism in bone fracture. *Journal of pharmaceutical sciences and research. 7*(7):441.

14-Einhorn TA, Gerstenfeld LC. 2015. Fracture healing: mechanisms and interventions. Nature *Reviews Rheumatology. 11*(1):45-54.

15-Egawa S, Miura S, Yokoyama H, Endo T, Tamura K. 2014. Growth and differentiation of a long bone in limb development, repair and regeneration. *Development, growth & differentiation. 56*(5):410-424.

16-Thompson EM, Matsiko A, Farrell E, Kelly DJ, O'Brien FJ. 2015. Recapitulating endochondral ossification: a promising route to in vivo bone regeneration. *Journal of tissue engineering and regenerative medicine. 9*(8):889-902 .

17-Maes C, Carmeliet P, Moermans K, Stockmans I, Smets N, Collen D, Bouillon R, Carmeliet G. 2002. Impaired angiogenesis and endochondral bone formation in mice lacking the vascular endothelial growth factor isoforms VEGF164 and VEGF188. *Mechanisms of development. 111*(1-2):61-73.

18-Gerstenfeld LC, Cullinane DM, Barnes GL, Graves DT, Einhorn TA. 2003. Fracture healing as a post-natal developmental process: molecular, spatial, and temporal aspects of its regulation. *Journal of cellular biochemistry. 88*(5):873-884.

19-Fu R, Feng Y, Liu Y, Yang H. 2021. Mechanical regulation of bone regeneration during distraction osteogenesis. *Medicine in novel technology and devices. 11*:100077

دراسة تأثير هيالورونات الصوديوم والفيبرين الغني بالصفائح الدموية الذاتية على شفاء كسور الارتفاق في القطط

ابراهيم عبد الزهراء عباس[1]، لؤي احمد نعي[1]2، حسان محمد التميمي[1]
1- قسم الجراحة البيطرية والتوليد، كلية الطب البيطري، جامعة البصرة، العراق.

الخلاصة

بحثت هذه الدراسة في تأثير هيالورونات الصوديوم والفيبرين الغني بالصفيحات الدموية على عملية الشفاء في كسور الارتفاق في القطط. لإجراء هذه الدراسة تم استخدام 30 قطاً ذكراً بالغاً وتم تقسيمهم إلى ثلاث مجموعات (10 قطط لكل مجموعة). تتألف المجموعات من مجموعة السيطرة (بدون علاج)، ومجموعة هيالورونات الصوديوم (تُعالج بجل هيالورونات الصوديوم في موقع الكسر)، وهيالورونات الصوديوم مع مجموعة الفيبرين الغنية بالصفيحات الدموية (تُعالج بخليط من جل هيالورونات الصوديوم و الفيبرين الغني بالصفائح الدموية في موقع الكسر). تم إحداث كسور الارتفاق بواسطة المشرط الجراحي، تم تثبيت الكسور باستخدام سلك التطويق. تمت ملاحظة القطط سريريًا في الأيام الأول والثاني والثالث والسابع بعد الجراحة، وتم إجراء التقييمات النسيجية المرضية بعد 42 يومًا من الجراحة. أظهرت الدراسة تحسنا في اختفاء التورم في منطقة الكسر لدى المجموعات المعالجة. ومن المثير للاهتمام لوحظ أن القطط تستعيد استخدام فكيها بعد ثلاثة أيام فقط من الجراحة، وهو ما لم يحدث في المجموعة السيطرة. تشير النتائج النسيجية إلى شفاء الكسور بالكامل في مجموعة هيالورونات الصوديوم و الفيبرين الغني بالصفائح الدموية (SH&PRF)، مع إعادة تشكيل العظام بشكل نشط، وتكوين الكالس الغضروفي، وتفاعل ليفي محيطي دون التهاب. أظهرت مجموعة SH&PRF خلايا غضروفية وتعظم داخل الغضروف أكثر نشاطًا من مجموعات SH ومجموعة السيطرة، مما يشير إلى نتائج أفضل لإصلاح العظام. تشير الدراسة إلى أن هيالورونات الصوديوم ومركب الفيبرين الغني بالصفائح الدموية يعززان ويسرعان عملية التئام الجروح بشكل فعال.

الكلمات المفتاحية: القطة، كسر ارتفاق الفك السفلي، هيالورونات الصوديوم، الصفائح الدموية.