Research Article

BASJVETRES

BASRAH JOURNAL OF VETERINARY RESEARCH, 2024, 23(3):106-117
https://bjvr.uobasrah.edu.iq/

Histopathological Changes of Applying Nano-Fat on Articular Cartilage Degenerative Defect in A Dog Model

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DOI: https://doi.org/10.23975/bjvr.2024.150398.1093

Received: 6 June 2024 Accepted: 17 July 2024.

Abstract

Damage to articular cartilage can ultimately lead to osteoarthritis, a debilitating degenerative joint disease. Treating cartilage damage represents a real challenge in orthopedic surgery because cartilage has a limited ability to heal, and currently available treatments are limited. . This study was conducted to examine histological changes after using the nano-fat on the healing process of defective cartilage in dogs. The current study used ten healthy adult mongrel dogs, divided them into two equal groups (n = 5), and left Group I (the control group) without any treatment. Group II, known as the nano-fat group, received treatment by applying nano-fat. The results indicated that the peripheral portion, particularly the edges near the trochlear groove of the joint, shows signs of mineralization, while the operation site of the control group is filled with collagen fibers and numerous newly generated blood vessels. The control group did not experience cartilage regeneration. Nano-fat used as a treatment can effectively enhance articular cartilage regeneration, enhance the newly generated blood vessels, and accelerate the inflammatory process.

Keywords: articular cartilage, articular cartilage degenerative defect, Nano-fat, dogs' osteoarthritis.

Introduction

The articular cartilage is a connective tissue with significant mechanical durability and elasticity function (1). Because it naturally doesn't have many blood vessels, cartilage can't repair itself as well. This means that chondrocytes, which are cartilage's main cell, can't copy themselves as well (2). Injury can vary from sudden abnormalities, which, if left untreated, might lead to osteoarthritis (OA), to widespread cartilage deterioration in advanced stages of the illness (3). Furthermore, cartilage has a significantly restricted ability to mend naturally because it lacks blood vessels, has a complex structure, and its cells have a limited ability to replicate (4). Adipose tissue is a good source of progenitor cells because it contains relatively large amounts of MSCs, pericyte, and fibroblast compared to other sources, such as bone marrow, amniotic cells, umbilical cord, and placental tissue. (5). Nano-fat is the mixture of cells originating from adipose tissue, through mechanical or enzyme digestion, centrifugation, and filtration (6). This solution lacks adipocytes but encompasses diverse cells such as MSCs, stromal cells, pericytes, endothelial progenitor (EPC) cell populations, and immune components. Nano-fat is a mixture of cells obtained from abdominal adipose tissue. In order to get nano fats, the process will involve the use of centrifugation, mechanical digestion, and enzyme filtration (7). Nano-fats are autologous, easily available, and have few side effects, so their clinical applications have received much attention (8). However, nano-fats cannot be stored and have a very brief shelf life (9). Based on this knowledge, we examined the effectiveness of nano-fat in the healing defects of the articular cartilage of the dog's stifle joint. The success of this study may have significant clinical implications for the patients in future.

Materials and Methods

Experimental animals design

Used ten healthy mongrel dogs aged between 1-2 years old. with weight average of 20 kg. Animals were accoderated in separated cages. The Ethics Committee of the College of Veterinary Medicine, University of Basrah, approved all procedures used in this study, in accordance with License No. 42/2023. The animals were divided into two equal groups (n = 5). Group I (the control group) was left without treatment. While group II (the nano-fat group) was treated by the application of nano-fat. The dogs were euthanized at 56 days post-surgery.

Preparation of Nano fat

With some modifications, adipose tissue preparation was performed. We collected the adipose tissue, as shown in figure (1). The adipose tissue was mincing into small fragments by Metzenbaum scissors, then put into a petri dish with serum-free minimum essential medium (MEM) and 500µg/ml ampicillin and streptomycin as supplements. In a sterilising environment, the tissues were cleaned four times in phosphate-buffered saline (PBS) and suspended in PBS supplemented with 0.1% collagenase type II. The cells were then preheated to 37 ºC and incubated for 45 minutes at that temperature while being shaken. To halt the enzymatic

reaction, we added FBS and 10% MEM to the cell suspension figure (2). The sample underwent filtration through a 100-m nylon mesh filter to remove any cell debris. Then, centrifugation was carried out for ten minutes at 1000 rpm (figure 3). The pellets, which included the cell suspension, were next cultivated in a growth medium made of MEM with 20% fetal bovine serum (FBS) (figure 4). In 25 cm cell culture flasks, the cells were cultivated under 95% humidified air at 37 C degrees (10).

Cell culture

In a humidified environment at 37 °C, the cells were cultivated in MEM (Capricorn, Germany) supplemented with 10% foetal bovine serum (FBS) (Capricorn, Germany) and 100 IU penicillin-streptomycin (broad spectrum antibiotic). The cells were then examined under an inverted microscope (11).

Cell staining

Dissolve 0.5 g of crystal violet powder in 80 mL of distilled water with 20 mL of methanol. Next, store the solution in the dark at room temperature. Then, aspirate the medium (MEM, FBS and anti-biotic) from the cells, and wash the cells twice with (PBS). The researchers I used 5 mL of dye to stain the cells for 20 min. After aspirating the stain and washing it with water, I examined the cells under an inverted microscope. (12). Under a microscope, the main constituents are mature adipocytes and nano-fat cells, as shown in figures 5 and 6 (13).

Surgical procedure

Dogs were anaesthetized using a combination of 2% xylazine hydrochloride and 10% ketamine hydrochloride at a dose of 5 mg/kg BW I/M and 10 mg/kg BW I/M, respectively (14). All dogs underwent a defect 4 mm deep and 8 mm diameter that was induced in the trochlear groove of the distal part of the left femoral bone by using a manual drill. The incision extended longitudinally from the proximal end of the patella to the insertion of the patellar ligament on the tibial tuberosity. The stifle was flexed, and then the patella and joined tissues were luxated medially to provide access to the distal femoral joint surface. The trochlear exposed, and the defect point was selected in the non-weight-bearing area in the center of the patellar groove. Then, the dogs were prepared for nano-fat application, after which the joint was prepared aseptically. The nano-fat was then injected into the trochlear groove figure (7).. The incision was then closed as usual.

Histopathological evaluation

After euthanasia by overdose anesthesia, we promptly incised the stifle joint and collected samples of the chondral tissue at the procedure site. We used a medical saw to separate the defect area at the center of the patellar groove from the stifle joint.Then, the samples have been maintained with a solution of neutral buffed formalin, which has a concentration of 10%. Subsequently, the specimens underwent sectioning and paraffin embedding. The sagittal slices were cut and stained with haematoxylin and eosin. The sections were microscopically assessed by a pathologist (15).

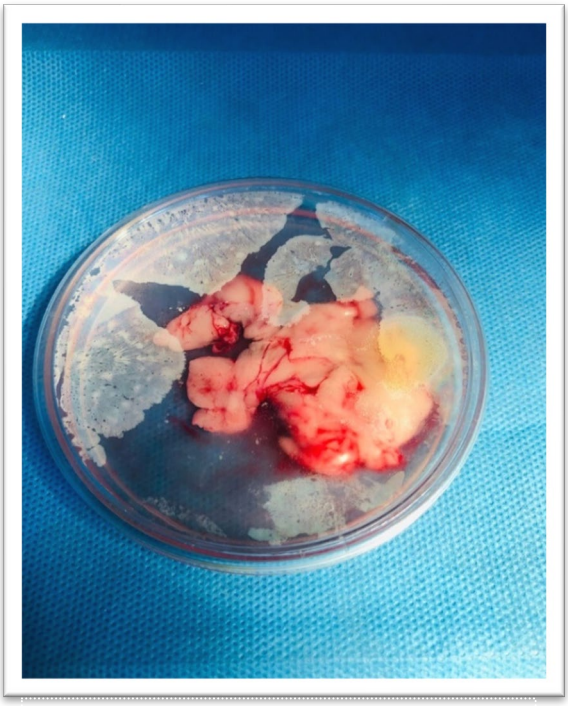

Fig. 1: shows collection of adipose

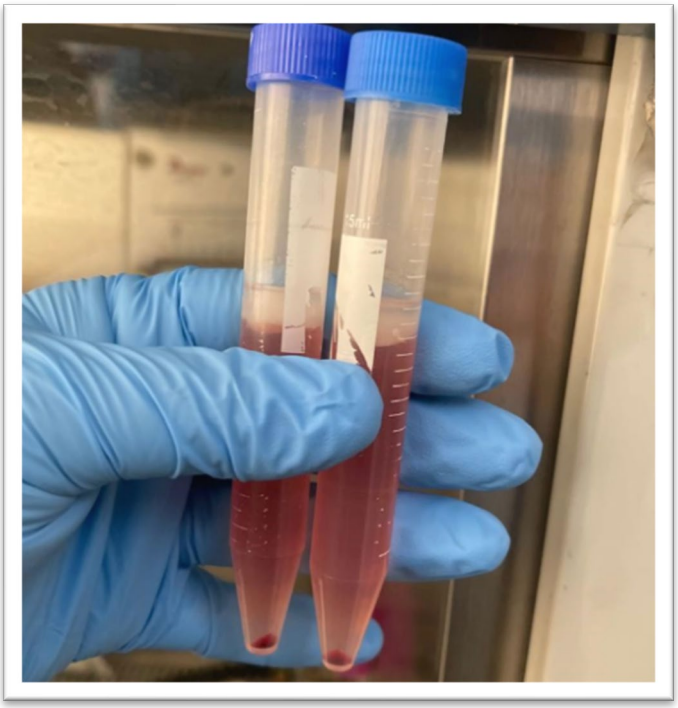

Fig. 2: shown adding FBS 10%

Fig. 3: shows centrifugation of adipose tissue at 1000 rpm for 10 minutes

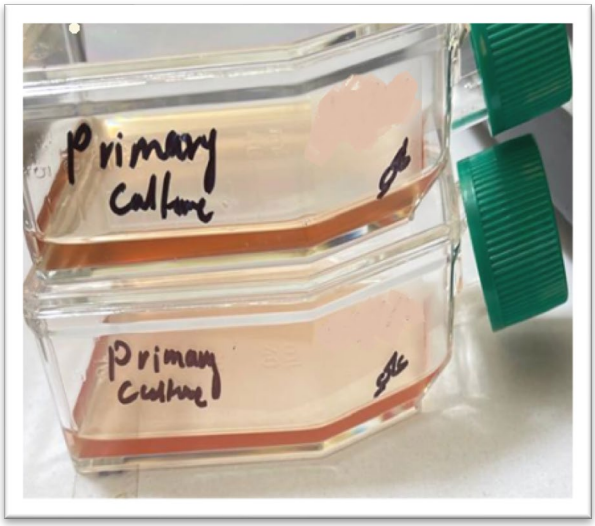

Fig. 4: shows culturing of the cells in a growth media

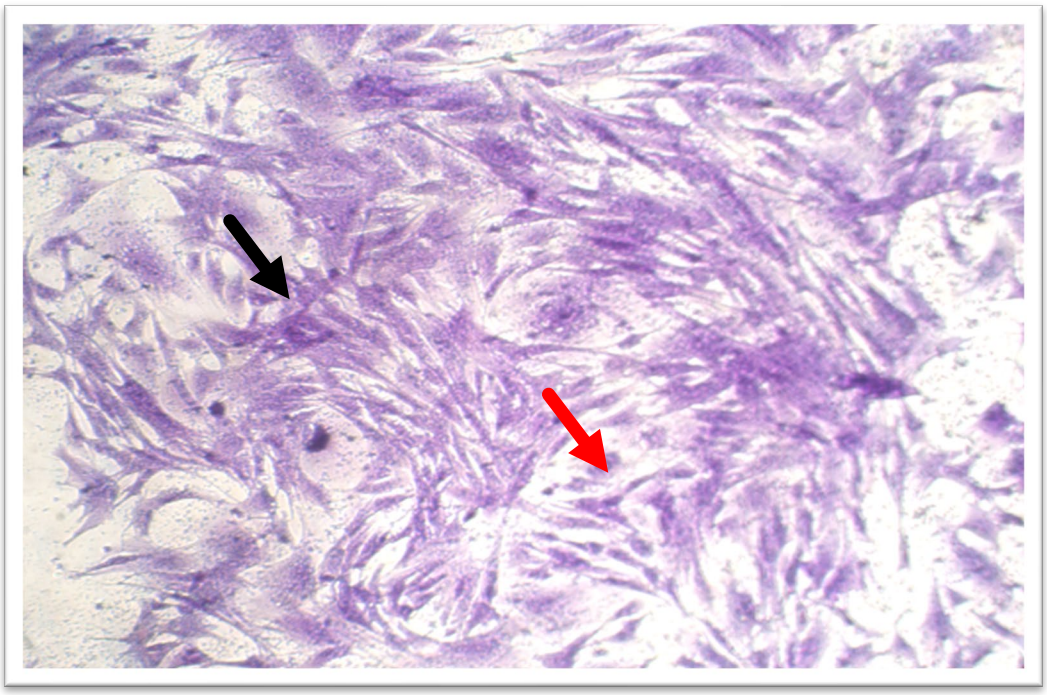

Fig.5: shows cells of Nano-fat (fibroblast cells (red arrow), mesenchymal stem cells (black arrow))

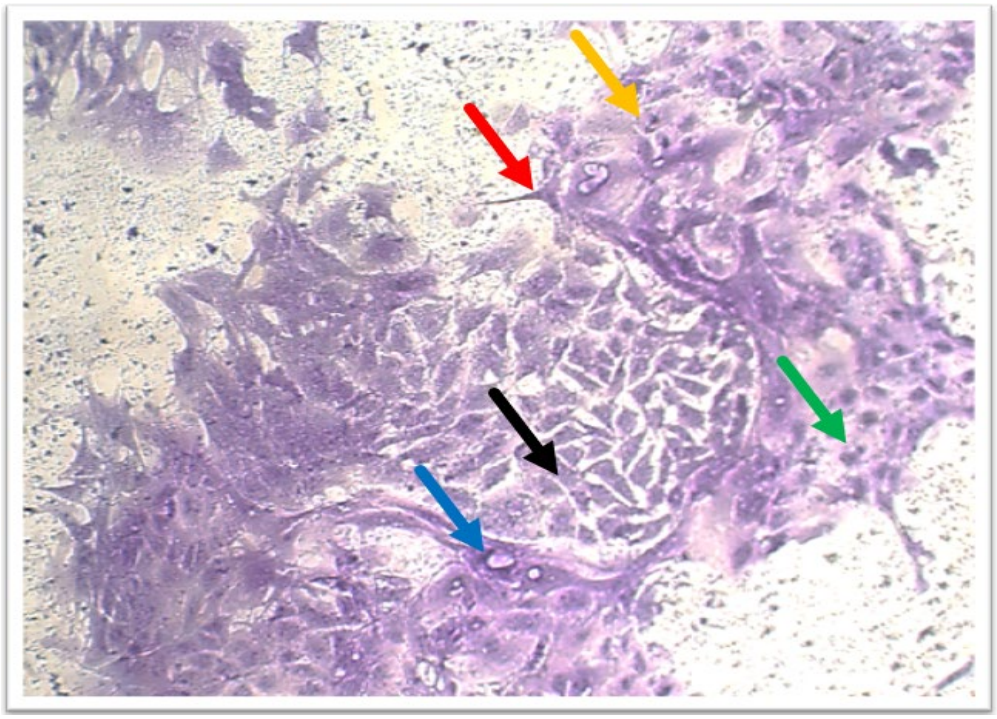

Fig. 6: shows fibroblast (red arrow), mesenchymal stem cells (black arrow), adipocyte (blue arrow), pericyte (green arrow) and immune cells (yellow arrow).

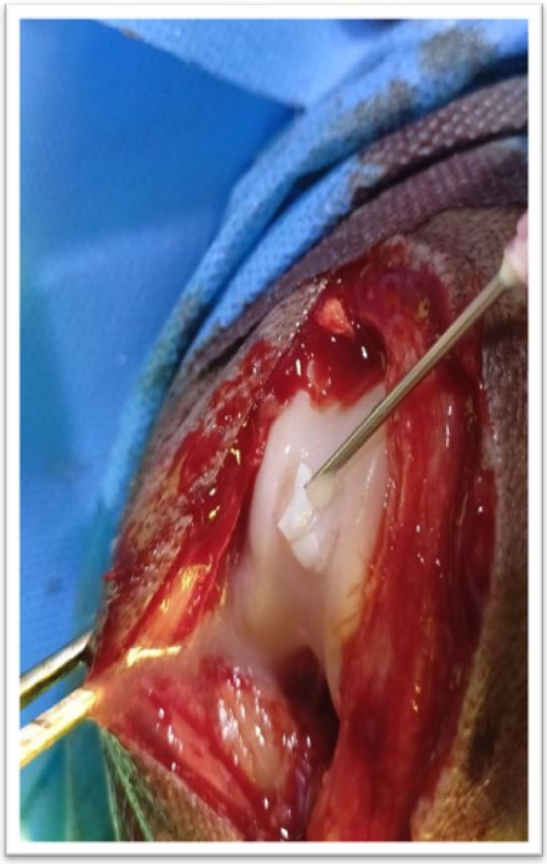

Fig. 7: shows application of nano-fat

Results

The control group results showed that the site of operation was filled with collagen fibers, and newly generated blood vessels were evident and numerous. However, this group did not include cartilage regeneration, as shown in figures (8).

Nano-fat group results showed marked cartilage regeneration at the operation area; the regenerated cartilage was well differentiated. The area is filled with fully mature cartilage. The control group results showed that the site of operation was filled with collagen fibers, and newly generated blood vessels were evident and numerous. However, this group did not include cartilage regeneration, as shown in figure (9)

Discussion

Articular cartilage is a specialized tissue that provides smooth and low-friction surfaces for joints. Even so, the integrity of the object might be compromised due to trauma, extreme load, aging, and inflammation (16). Due to its lack of very low blood supply and nutrition supply, it cannot repair and regenerate itself, as pointed out in (17). Osteoarthritis is a common progressive disease in dogs, particularly in large breeds. Osteoarthritis is also defined as a degenerative joint condition characterized by cartilage degeneration caused by aging, injury, repetitive stress, or disease (18).

Osteoarthritis can affect any joint in the body, but it primarily affects limbs that have been diagnosed with the condition (19). In this paper, the first group (control) had a strong inflammatory response at the grafting site, showing signs of angiogenesis and mild collagen fiber deposition. This outcome indicates that the healing process is still at the beginning, according to (20), where the inflammatory process is still active, and the newly formed vasculature occurs in response to the inflammation (1). The Nano-fat showed superior results in cartilage development and reduction and accelerated of the inflammatory process in the grafting site (6), marked cartilage regeneration appears to fill all the defect sites; few fibrous tissues remain. The area is filled with fully mature cartilage (21).

It was the same as what was shown by (22), who said that nano-fat tissue can be used to treat joint damage and degenerative osteoarthritis because it contains collagens I, III, IV, VI, and VI, laminin, elastin, fibronectin, vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF). Furthermore, nano-fat not only enhances the adipogenic potential of adipose mesenchymal cells, but also stimulates and accelerates the differentiation of stem cells (23). The results also were mention by (24), who reported that adipose tissue contains large amounts of bioactive ECM constituents and progenitor cells that can promote adipogenesis and angiogenesis that have an important role in active chondrocyte, differentiation chondroblast, and regenerative cartilage.

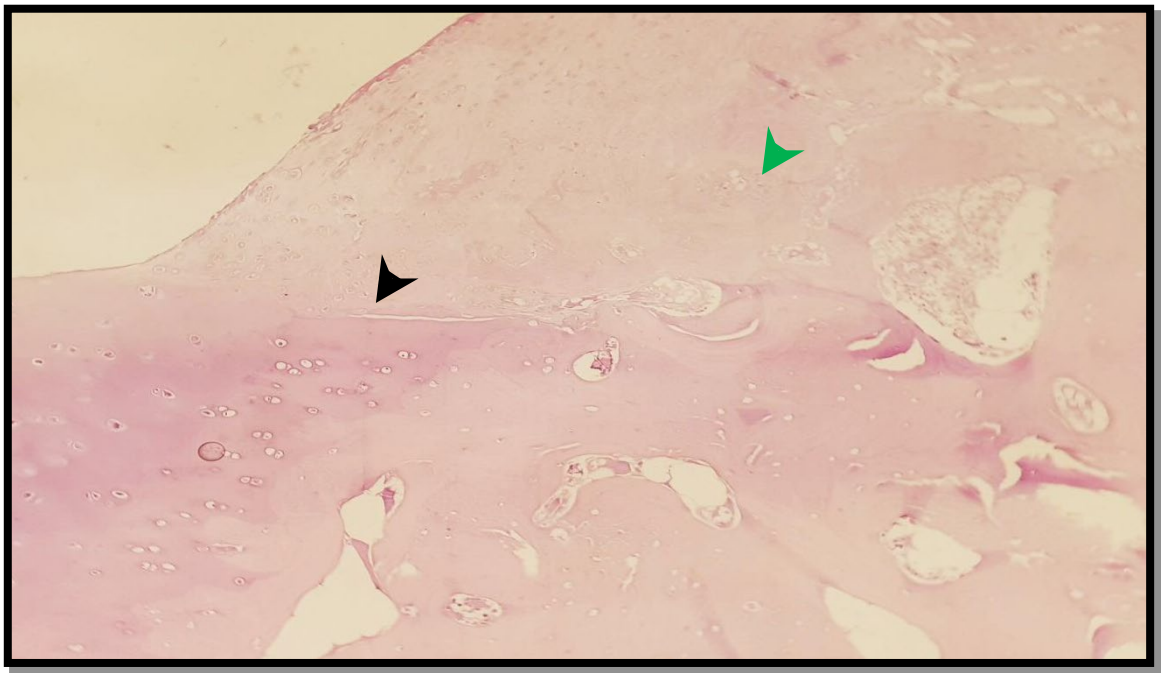

Fig.8 (A): shows articular surface at the site of cartilage damage (control group) shows well demarcated line between the original cartilage and the operation site (black arrow) chondrocyte differentiation in the operation site (green arrow) H&E 10X.

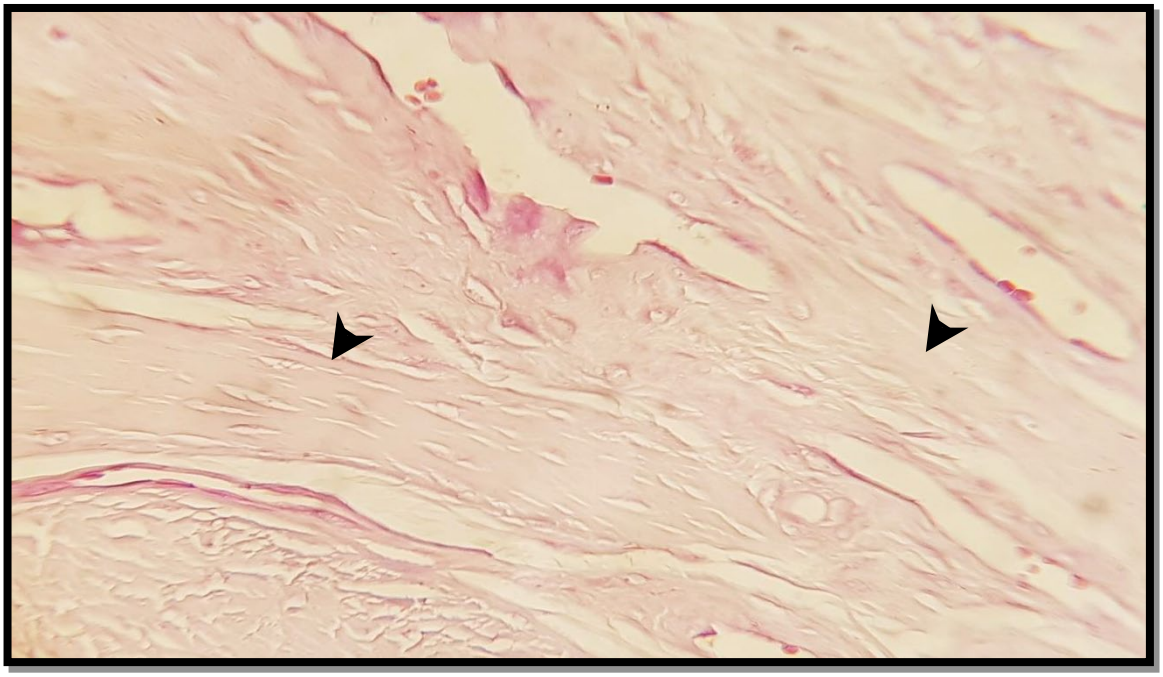

Fig.8 (B): shows articular surface at the site of cartilage damage (control group) shows intensive collagen ingrowth in the site of operation (black arrow). H&E 40X

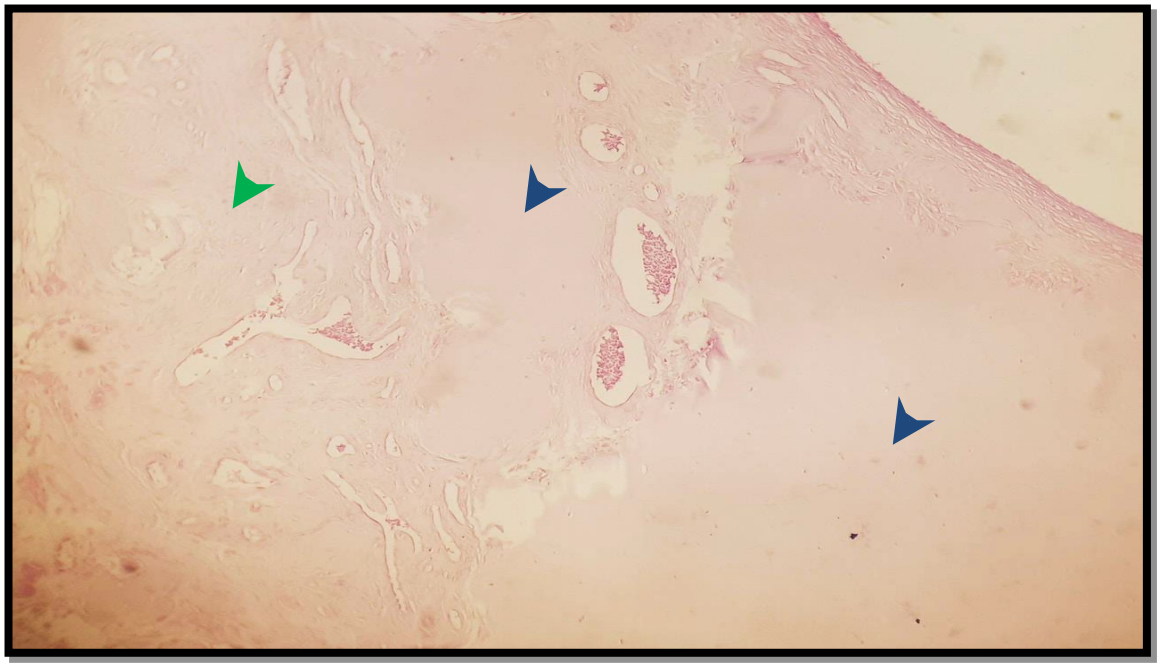

Fig.9 (A): shows articular surface at the site of cartilage damage (Nano-fat group) shows marked fibrous tissue deposition in the operation site (green arrow) area of structureless material (blue arrow). H&E 4X

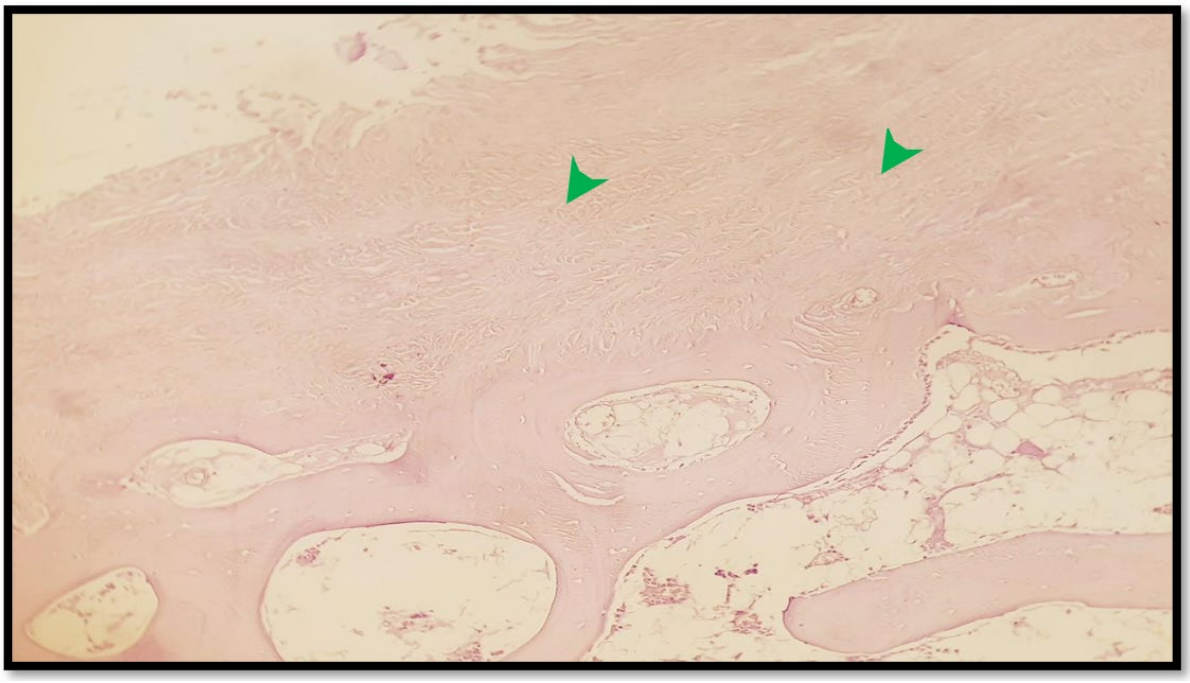

Fig.9 (B): shows articular surface at the site of cartilage damage (Nano-fat group) shows marked fibrous tissue deposition in the margin of operation site (green arrow). H&E 40X

Conclusion

The current study showed that nano-fat was an effective therapy for stopping the breakdown of articular cartilage in dogs with osteoarthritis. This is because nano-fat is easier to make and there are fewer ethical and safety concerns. All things considered, this study provides new insight into the anti-articular cartilage degrading effectiveness of nano-fat and suggests that it is a viable and effective therapy option for osteoarthritis treatment.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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التغيرات النسيجية المرضية لتطبيق الدهون النانوية الذاتية على ضرر الغضروف المفصلي التنكسي في الكلاب.

علي وصفي صادق و باسم كاظم خشجوري.

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الخلاصة

يمكن أن يؤدي تلف الغضروف المفصلي في النهاية إلى التهاب المفاصل التنكسي، وهو مرض تنكسي موهن. إن قدرة الشفاء الطبيعية المحدودة للغضروف والعلاجات الفعالة المحددة حاليا تجعل علاج ضرر الغضروف أمرا صعبا. نفير النسيج الدهني، وهي نوع معين من الأنسجة الدهنية، تضم أكبر تركيز من الخلايا الجذعية الوسطية، والخلايا المولدة للخلايا الليفية، والخلايا المولدة للخلايا المحيطة. أجريت هذه الدراسة لتقييم فعالية الدهون النانوية الذاتية في شفاء الغضروف المفصلي في الكلاب . تم استخدام عشرة كلاب هجينة بالغة ذات صحة جيدة في الدراسة الحالية، قسمت الكلاب إلى مجموعتين متساويتين (م = 5)، المجموعة الأولى (مجموعة السيطرة) تركت دون علاج. بينما تم علاج المجموعة الثانية (مجموعة الدهون النانوية الذاتية) عن طريق وضعها في مكان الضرر. أظهرت النتائج أن علامات ترسب المعادن تحدث في الجزء المحيطي، وخاصة في الحواف بالقرب من الأخدود البكري للمفصل، وتكون الكولاجين بشكل ملحوظ وتسريع عملية تكوين الخلايا الغضروفية مقارنة بمجموعة السيطرة. يمكن للدهون النانوية الذاتية المستخدمة كعلاج أن نعزز بشكل فعال تجديد الغضروف المفصلي، تعزز الأوعية الدموية المتولدة حديثا وتسرع عملية الالتهاب.

الكلمات المفتاحية: الغضروف المفصلي، ضرر الغضروف المفصلي التنكسي، الدهون النانوية الذاتية، التهاب المفاصل التنكسي في الكلاب.