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Original research paper

Ameliorative Effect of PRF and Silver Hydroxyapatite Nanoparticles bio-composite on femoral head AVN in a Rat Model

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ABSTRACT

Aim: The present study evaluates the specific effects of silver Nanoparticles (AgNPs) and silver hydroxyapatite nanoparticles (HA/AgNPs) combined with Platelets rich fibrin (PRF) for management of early stage of AVN in rats and their impact on hindering of AVN progression.

Animals: one hundred-twenty Sprague Dawley rats were divided into 5 groups.

Procedures: AVN was surgically induced by deprivation of the blood flow using bipolar electrocoagulation. AgNPs and HA/AgNPs were delivered to the ischemic femoral head through core decompression (CD) tunnel then sealed with PRF gel in each treated group of AgNPs and HA/AgNPs respectively. The healing was assessed clinically, grossly, radiographically with Cone beam computed tomography (CBCT) and histopathologically at 2, 4 and 8 weeks postoperatively.

Results: PRF-HA/AgNPs bio-composite improved pain sensation as proved by significantly enhanced mechanical hyperalgesia compared to both AVN, CD ($P < 0.0001$) and CD-PRF-AgNPs group ($P = 0.003$), it maintained head sphericity with restoration of joint mobility and validated increased bone mineral density (BMD) relative to both CD ($P = 0.0001$) and CD-PRF-AgNPs group ($P = 0.009$) and complete obliteration of CD tunnel than the other groups as indicated by CBCT evaluations and gross images. Conversely, AVN group displayed subchondral collapse followed by full degeneration of the head articular surface with the least CBCT records at 8 weeks ($P < 0.0001$). The histopathological measurements also revealed the greatest number of empty lacunae in AVN group with trabecular and epiphyseal thinning, however, CD-PRF-HA/AgNPs group significantly recorded the lowest number of empty lacunae compared to other treated groups at each time ($P < 0.0001$) with restoration of normal osteoid tissue, it also enhanced bone matrix deposition and extracellular matrix restoration which were confirmed by Masson Trichrome staining, while the mineralization was shown by Alizarin s red staining with the strongest immunohistochemical expression of OPN and CD34 compared to the other groups at each time point ($P < 0.0001$).

Conclusion: The present results indicated that HA/AgNPs combined with PRF gel administered locally in ischemic femoral head stimulated osteogenesis and angiogenesis, contributing to enhanced protection and repair of the femoral head, guarded against accelerated bone turnover, restored of joint stability and provided promising therapy during the early stage of AVN.

Key words: Avascular necrosis, Cone beam computed tomography, Core decompression, Silver nanoparticles, Silver hydroxyapatite nanoparticles, Platelets rich fibrin, Rat

1. INTRODUCTION

AVN, also called Legg-Calve-Perthes disease occurs when blood flow to the femoral head is limited. This causes bone cells to die, it mainly affects weight-bearing joints like the hip and knee causing pain, joint dysfunction. AVN may be traumatic including fracture of the femoral neck and hip dislocation ,or non-traumatic including thrombosis due to corticosteroid abuse, radiation therapy, malignancy and genetic factors [1]. The exact pathogenesis of AVN is often imprecise [2].

AVN often leads to the gradual collapse of the femoral head, which progresses to osteoarthritis. The patient will frequently need total hip replacement (THA) as the osteoarthritis progresses [3]. As a result, early detection of AVN gives physicians alternatives to THA. Numerous imaging methods, such as computed tomography (CT), magnetic resonance imaging and X-rays, were found to be useful in identifying the signs of bone necrosis. Subchondral collapse is indicated by subchondral radiolucency, known as the "crescent sign," on a radiographic examination [4].

CBCT, a recently developed CT modality, uses a cone X-ray beam rather than the traditional fan-beam; Because of its advantages over traditional CT, such as its higher spatial resolution and comparatively lower cost and radiation dose. It is frequently used for the clinical diagnosis of dental complications [5, 6]. In CBCT scanning, different bone densities in different sites can be represented and compared by the Hounsfield units attenuation measures [7].

The goal of early-stage AVN therapy is to avoid subchondral collapse. Restricted weight-bearing and pharmaceutical or biophysical therapy are examples of non-operative approaches, although CD is a frequent surgical technique. Despite its effectiveness, CD increases the risk of fracture by weakening the femoral head. Recently, combining CD with bone grafting is widely practiced [8]. Recent developments in the treatment of osteonecrosis emphasize the move toward localized therapies from systemic therapy, which has drawbacks such as inadequate local concentration and adverse consequences. For focused treatment, they include infusing growth factors, cytokines, medications, hormones, stem cells, and other substances straight into the afflicted area. Significant advancements have also been made in bone tissue engineering, which highlights the part

angiogenesis plays in bone healing. By combining growth factors and CD, this method seeks to improve bone healing, protect the femoral head, and maybe do away with the necessity for hip replacement surgery [9].

PRF was thought to be the second generation of platelet concentrates that functions as a healing biomaterial because it is rich in platelets, fibrin; leukocytes; circulating stem cells; healing cytokines; the pro-inflammatory cytokines interleukin-1 β , interleukin-6, and tumor necrosis factor- α ; the anti-inflammatory cytokines interleukin-4 and interleukin-10; vascular endothelial growth factor; insulin-like growth factor-I and II; epidermal growth factor, transforming growth factor- β 1 and platelet-derived growth factor [10, 11].

Although the growth factors show potential for angiogenesis and osteogenesis, they are unable to support the collapsing femoral head. Therefore, combining growth factors with injectable material systems may result in a therapeutic system that enhances regeneration and mechanical support for the collapsed femoral head [8].

Nanotechnology has advanced bone tissue engineering. The need for biomaterials that closely resemble the inorganic hydroxyapatite crystals (HA) and organic matrix found in native bone is highlighted. [12].

HA, particularly in nanoscale forms, is a highly biocompatible ceramic that is frequently utilized in orthopedic biomaterial due to its excellent biocompatibility, bioactivity, and osteoconductivity. To replicate the natural bone mineral phase, HA nanoparticles are used to mineralize organic matrices [13].

One of the main reasons for implant failure in orthopedic surgery is infection. This is countered by combining AgNPs, which have osteoinductive and antimicrobial effects, with HA scaffolds. By affecting osteoblast activity, AgNPs promote bone repair and guard against infections. Orthopedic implants benefit greatly from the superior biocompatibility, osteoconductivity, biodegradability, and mechanical strength of silver-based HA bio-composites [14].

2. MATERIALS AND METHODS

Animals, housing and feeding

A total of one hundred-twenty male Sprague-Dawley (SD) rats weighing between 250 and 350 grams participated in this study. Rats were bred, sourced and housed at the animal housing of the Medical Experimental Research Center (MERC), Faculty of Medicine, Mansoura University, at

temperatures between 20°C and 25°C. They had unrestricted access to food and water and were housed in stable conditions. The Mansoura University Faculty of Medicine's Local Ethical Committee accepted the experimental protocol for this study in accordance with the National Research Center-Egypt's Ethics Committee, which registered it under registration number (Ph.D /105).

Study design

Sample size calculation was based on Epidermal growth factor (EGF) effect on promotion of bone healing in surgically induced osteonecrosis of the femoral head retrieved from the most similar research [15]. Using G power program version 3.1.9.7 to calculate sample size based on expected effect size of 1.68 (163.33 ± 20.77 & 192.5 ± 13.17) based on subchondral distance ,using 2-tailed test , α error =0.05 and power = 80.0% , the total calculated sample size will be 7 in each group at least . The present study was approved following the ethics of the Mansoura University Animal Care and Use Committee (Ph.D/105) which met the ARRIVE guidelines. All methods were carried out in accordance with relevant guidelines and regulations.

All rats were divided into 5 groups; group (C-ve) negative control divided in to 3 subgroups (2,4 and 8 weeks) each subgroup (n = 8 rats), group (AVN) rats were subjected to surgical induction of AVN divided in to 3 subgroups (2,4 and 8 weeks) each subgroup (n=8 rats), group (CD) AVN rats were treated with core decompression divided in to 3 subgroups (2,4 and 8 weeks) each subgroup (n = 8 rats), group (CD-PRF-AgNPs) AVN rats were treated with PRF-AgNPs composite divided in to 3 subgroups (2,4 and 8 weeks) each subgroup (n=8 rats),and group (CD-PRF-HA/AgNPs) AVN rats were treated with PRF- HA/AgNPs composite divided in to 3 subgroups (2,4 and 8 weeks) each subgroup (n=8 rats).

Anesthesia and surgery

All rats were anaesthetized by intra-peritoneal injection of a mixture Xylazine Hcl in dose 10 mg/kg (XYLA-JECT (20mg/ml), ADWIA pharmaceuticals Egyptian company, New Cairo,

Egypt) and ketamine Hcl in dose 75mg/kg (Ketamax (50mg/ml),Troikaa Pharmaceuticals Ltd, Gujarat, India) [16].

All SD rats were anesthetized and placed on a warmed operating pad. following aseptic preparation, trochanter was aseptically prepared, a longitudinal incision was made over the greater trochanter, the gluteus muscles were split and detached from the bone, the articular capsule was transected, the femoral head was dislocated from the acetabulum, and the Ligamentum Teres was cut. The blood vessel around the femoral neck was deprived circumferentially by using bipolar electrocoagulation (KLS Martin, Germany) to induce AVN. After relocation of the femoral head, the skin and muscles were sutured one after the other as shown in (Fig.1) [17].

Preparation of platelet rich fibrin (PRF) gel

Platelet-Rich Fibrin (PRF) preparation was carried out using a one-step centrifugation method (Centurion Scientific centrifuge, K2015, no. 12778-1, UK). A 10 ml blood sample was collected from the donor rat via cardiac puncture, as described by [18]. This technique was selected to obtain a sufficient PRF membrane from a single donor rat to support two experiments using PRF gel. The harvested blood was placed into a sterile 5 ml vacuumed plain glass tube (EL Dawlia International Co., Asyut, Egypt) at a rate of 3000 rpm for 10min (RCF=402×g) at a 45° rotor angulation with a radius of 40mm. With special attention to PRF-bone contact, the obtained PRF gel was carefully applied to the femoral head [19]. In all treated groups, CD of the femoral head was accomplished by a 21-gauge needle in a rotating manner starting from the insertion point of the Ligamentum Teres (center of the femoral head) through the head and the neck [20]. Single dose of AgNPs [21, 22]. and HA/AgNPs [23] was intraosseous injected by a 28 G needle through the CD tunnel then the tunnel was sealed with PRF gel, the muscles and skin were stitched as usual (Fig.2).

To maintain body temperature and monitor any negative complications, the rats were placed in a recovery room under heat lamps for around two hours following surgery. Each rat was given amoxicillin (E-MOX, EIPICO, Egypt) in dose (20 mg/kg IM), and analgesic Meloxicam (MOBITIL, MUP, Egypt) in dose (5 mg/kg SC) once a day for the first 3 days, and once animals regained complete consciousness, were allowed free cage activity until the end of the study.

Evaluation of AVN

Clinical assessment

Pain was subjectively evaluated by observing changes in locomotor activity, such as avoiding weight-bearing on an ischemic limb, along with observing food and water intake and body weight [24].

Mechanical hyperalgesia was measured using a Randall–Sellito algesiometer, which applies increasing pressure to the left hind paw to determine the nociceptive threshold. The threshold, recorded as the force in grams required to induce a pain response (vocalization or vigorous struggle). The nociceptive threshold (gram) was recorded at the 2-, 4- and 8-weeks post-surgery and compared to the C-ve group [25].

Gross evaluation

Animals at 2,4 and 8 weeks after surgery were sacrificed using an overdose of thiopental sodium anesthesia in dose (150 mg/kg) administered by intraperitoneal injection [26]. All animals were anesthetized and made unconscious before euthanasia. A high dose of thiopental sodium was administered during the euthanasia procedure to ensure a quick loss of consciousness and subsequent cardiac arrest. This method was selected based on ethical considerations to reduce pain and suffering. Following euthanasia, the left femoral heads were photographed and subjected to macroscopic examination by two blinded examiners who compared them to the C-ve group to evaluate the degree of head's sphericity preservation and degenerative alterations [27, 28].

Radiographical evaluation

The course of bone healing during a 60-day postoperative period was assessed using both quantitative and qualitative criteria. An orthodontic-grade CBCT scanner (120kV, 5mA; i-CAT FLX V17–19; Imaging Sciences International, Hatfield, USA) with a clinically standard 0.125-mm voxel size was used to perform CBCT scans on the 14th, 30th, and 60th days following surgery. The traditional filtered back-projection approach was used to rebuild the 3D pictures from 2D projections. 3D volume renderings were created using the On-Demand 3D software program (version 1.0.7510; Cybermed, Korea), which provides visualization in multiplanar orthogonal perspectives (sagittal, axial, and coronal) [19].

The left femoral heads were harvested and fixed in 10% neutral buffered formalin. The bilateral anterior-posterior and lateral CBCT imaging to evaluate typical osteosclerotic changes, the crescent sign, subchondral bone collapse, loss of head sphericity, and late-stage degenerative changes in the AVN group according to the Steinberg scoring system [29] (Table;1). For the treated groups, the focus was on assessing femoral head preservation, prevention of collapse, and tunnel obliteration. The femoral heads were sorted according to the group number, and an image was taken for each group.

Deformity of the femoral head was quantified by the radiographic measurements of the femoral head were width (the widest diameter), and height (a vertical distance from the diameter line to the tip) and area on the anteroposterior radiograph of each rat and these measurements were taken by a blinded radiologist [15]. BMD of the original dense structure of the femoral head was measured using a quantitative CT method by Hounsfield units according to [30]. Due to the non-uniformity of the BMD in the same CT image, six-point densities were measured in the femoral head for each point and the mean $\pm$ SD was calculated. The mean of bone mineral densities of each group at each time (14th, 30th, and 60th PO days) was statistically compared with the total mean of the normal densities of the C-ve group [27].

Histopathological Assessment

The left femoral heads fixed in 4% PBS-buffered formaldehyde at 4° C for one day and decalcified in 10% PBS-buffered EDTA at 4° C for one month. Decalcified femoral heads were embedded in paraffin blocks, the tissues were longitudinally sectioned at 5 μ m and the histological sections were obtained at the midline of the femoral head, stained with hematoxylin and eosin (HE), Masson trichrome stain to evaluate the repair performance of necrotic femoral head [27, 28], and Alizarin Red S stain to evaluate the degree of calcification [31] (Gleeson et al., 2010) [32, 33]. Histological evaluations were conducted in a blind manner to ensure objectivity; the examiners were unaware of the treatment group allocations during the analysis. All light microscope images were captured at 100% magnification.

The sections of the left femoral heads were examined histopathologically to analyze Trabecular thickness, subchondral area, epiphysis thickness and the number of empty lacunae in the femoral head for each rat [15, 34] .

Immunohistochemical Analysis

Using immunohistochemistry, bone formation and vascular density were examined. Briefly, paraffin embedded tissues were deparaffinized, rehydrated and exposed to antigen retrieval citrate buffer in a microwave. Endogenous peroxidase activity was quenched with peroxidase blocking serum; slides were rinsed with PBS. Then, protein blocking serum were added followed by incubation with either of the following primary antibodies: OPN rabbit polyclonal antibody (catalogue number bs-0026R, BIOSS Biotechnology, Beijing, China, diluted 1:400) and CD34 rabbit monoclonal antibody (catalogue number A19015, ABclonal, Woburn, MA, United States, diluted 1:100). The sections were counterstained with Mayer's hematoxylin, carefully washed with distilled water, treated with ethanol and dried in xylene. Finally, the Sections were imaged at 400x and calculated the number of immune positive cells per 100 cells.

Statistical analysis

Data were fed to the computer and analyzed using Graph Pad Prism 8.0.2. The Shapiro-Wilk test was used to verify the normality of distribution. Quantitative data was described using mean and standard deviation. Normally distributed quantitative variables were analyzed using two-way ANOVA test followed by Post Hoc test Tukey test for multiple comparison. A P-value below 0.05 was considered significant.

3. RESULTS

Clinical findings

The study found that rats experienced pain, decreased activity, appetite, and pale eyes after surgery with diminished motion in operated hips, they recovered to normal activity within a week. The animals' postoperative course was uncomplicated. During 8-weeks observation period, some AVN and CD rats experienced weight loss.

In mechanical hyperalgesia, the CD-PRF-HA/AgNPs group estimated a significant improvement in the decreased nociceptive threshold compared to AVN ($P = 0.01$), CD ($P = 0.04$) with no significant difference compared to CD-PRF-AgNPs group at 2 weeks. It was significantly better than AVN ($P = 0.001$), CD group ($P = 0.02$) and CD-PRF-AgNPs ($P = 0.04$) in 4 weeks. Over 8 weeks, it still

showed an improvement compared to AVN, CD group ($P < 0.0001$) and CD-PRF-AgNPs ($P = 0.003$). However, it was significantly lower than C-ve at each time point. (**Table.2**).

Gross findings

Macroscopic observations of the femoral heads in AVN group displayed reservation of the head's sphericity at 2 weeks with a regular surface. By 4 weeks, they represented loss of their sphericity and flattening. At 8 weeks, they showed noticeable defect with complete loss of the articular surface and visible femoral neck resorption compared to C-ve group which had spherical, regular and glistening surface with normal femoral neck. CD group preserved their sphericity at 2 and 4 weeks. While, at 8 weeks, they clearly deformed and flattened with less prominent bony defects compared to the AVN group. On the other side, CD-PRF-AgNPs group reserved femoral head sphericity with homogeneity of grayish coloration and regular surface at 2 and 4 weeks. While, at 8 weeks, it showed slightly loss of the head sphericity with visible no obliteration of CD tunnel. The CD-PRF-HA/AgNPs group restored the normal appearance of the femoral head with spherical and smooth surface without any degenerative changes at each time point (**Fig.3**).

Radiographic findings

According to Steinberg score system, In AVN group, the sign of AVN started to appear 2 weeks after induction of ischemia which represented by subchondral collapse (crescent sign) with small amount of high density shadow (score 3) and preserved head sphericity, at 4 weeks, the femoral head had irregular hemispherical shape and uneven density with dark shadow and exhibited complete flattening with shortening of femoral neck (score 4). By 8 weeks, it exhibited resorption of the femoral head with advanced degenerative changes and complete loss of the articular surface (score 6) compared to C-ve group which had no evident of radiographical changes with spherical, regular shape and uniform density (score 0) (**Fig.4**).

CBCT imaging revealed difference in the healing of CD tunnel and architecture of the femoral head among treated groups, the femoral head in CD group preserved its spherical shape with no obliteration of the tunnel at 2 and 4 weeks, however, at 8 weeks, it showed clear signs of subchondral collapse and femoral neck resorption, with partial tunnel obliteration. The group treated with CD-PRF-AgNPs maintained the femoral head's sphericity, with the tunnel visible at 2 weeks and partially obliterated at 4 and 8 weeks. The femoral head of CD-PRF-HA/AgNPs group

had basically normal architecture with the newly mineralized tissue partially filling the CD tunnel at 2 and 4 weeks postoperatively and complete repair with full tunnel obliteration by 8 weeks (Fig.4).

Deformity of the femoral head was assessed through analysis of CBCT measurements, the width of femoral heads recorded no significant difference among all groups at 2 weeks. There was a significant increase in AVN group compared to all groups at 4 weeks ($P < 0.05$). Although, CD group was significantly greater than AVN group but it was still less than other groups at 8 weeks ($P < 0.0001$) (Fig.5).

The height and area of femoral heads didn't record any significant difference among all groups at 2 weeks. There was a significant decrease in AVN group compared to all groups in 4 weeks ($P < 0.001$). While, at 8 weeks, CD group was significantly higher than AVN group but still less than other groups ($P < 0.0001$) (Fig.5).

The radiographic measures of BMD showed no significant difference among all groups at 2 weeks. By 4 weeks, AVN group recorded a significant decrease compared to CD group ($P = 0.04$), CD-PRF-AgNPs group ($P = 0.006$), CD-PRF-HA/AgNPs ($P = 0.0008$). 8 weeks later, CD group was statistically less than CD-PRF-AgNPs group ($P = 0.002$), and CD-PRF-HA/AgNPs ($P = 0.0001$) with no significant difference compared to AVN group. While, CD-PRF-HA/AgNPs group recorded higher BMD than CD-PRF-AgNPs ($P = 0.009$) and there was no significant difference between CD-PRF-HA/AgNPs and C-ve groups (Fig.5).

Histopathological findings

The histological examination of the femoral heads within the AVN group demonstrated that Large empty spaces with the thinning and destruction of cortical and trabecular bone at 2 and 4 weeks, replaced by granulation tissue at 8 weeks. On the contrary, the C-ve group showed normal cortical bone with normal bone lacunae containing osteoblast, trabecular bone and osteoid matrix. After 2 weeks, CD group displayed inflammatory exudate filling cortical and trabecular gap followed by newly fibrovascular tissue in the subcortical area 4 weeks later and mature fibrous tissue replacing the cortical and trabecular bone at 8 weeks. In CD-PRF-HA/AgNPs group displayed restoration of the normal cortical and trabecular bone with normal osteoid and bone lacunae containing osteoblast at each time relative to CD-PRF-AgNPs group which displayed cortical bone has large

empty spaces at 2 and 4 weeks with destruction of cortical and trabecular bone replaced by fibrous tissue at 8 weeks (**Fig.6**).

Histopathological analysis in AVN group recorded the greatest number of empty lacunae which gradually increased by the time compared to all groups at each time point. On the contrary, the number of empty lacunae in CD-PRF-HA/AgNPs treated group showed a noticeable decrease compared to other treated groups but still higher than C-ve group at each time ($P < 0.0001$) (**Fig.7**).

The histological analysis of trabecular thickness in the AVN group showed marked decrease compared to all groups ($P < 0.0001$). While, the CD-PRF-AgNPs group estimated significant improvement compared to AVN and CD groups but still lower than CD-PRF-HA/AgNPs group ($P < 0.0001$) which had no significant difference relative to C-ve group at each time (**Fig.7**).

The statistical analysis of subchondral area of CD-PRF-HA/AgNPs showed significant improvement compared to all treated groups but still lower than C-ve groups at each time ($P < 0.0001$). At 2 and 4 weeks, CD group recorded a significant decrease compared to CD-PRF-AgNPs ($P < 0.0001$) and no significant difference compared to AVN group, while in 8 weeks, it was still less than CD-PRF-AgNPs group but greater than AVN group ($P < 0.05$) (**Fig.7**).

The histological analysis of epiphyseal thickness estimated no significant difference among AVN, CD and CD-PRF-AgNPs groups. On the other hand, CD-PRF-HA/AgNPs group was significantly higher than the other treated groups ($P < 0.0001$) with no significant difference compared to C-ve group at each time (**Fig.7**).

Masson Trichrome staining displayed gradually osteoid matrix loss in the AVN group, with moderate bluish staining at 2 weeks and mild staining at 4 and 8 weeks and showed notable deformity compared to C-ve group which displayed intense bluish staining. CD-PRF-HA/AgNPs group showed intense bluish- staining at each time. However, there was no obvious difference between CD and CD-PRF-AgNPs groups with moderate bluish- staining at each time (**Fig.8**).

Alizarin red S staining was applied for evaluating the degree of mineralization and displayed progressive depletion averaged between mild at 2 to no color at 8 weeks in both AVN and CD groups. CD-PRF-AgNPs group displayed moderate staining at 2 and 4 weeks and mild staining at 8 weeks revealing significant improvement compared to AVN and CD groups. While, CD-PRF-HA/AgNPs group displayed intense staining at 2, 4 weeks and moderate at 8 weeks (**Fig.9**).

Immunohistochemical findings

The AVN group exhibited the lowest levels of both OPN (**Fig.10**) and CD34 (**Fig.11**) expressions compared to the other groups. While, CD-PRF-AgNPs group exhibited significantly higher expression compared to AVN, CD groups but still lower than CD-PRF-HA/AgNPs group which displayed the greatest expression compared to all groups at each time ($P < 0.0001$) (**Fig.12**).

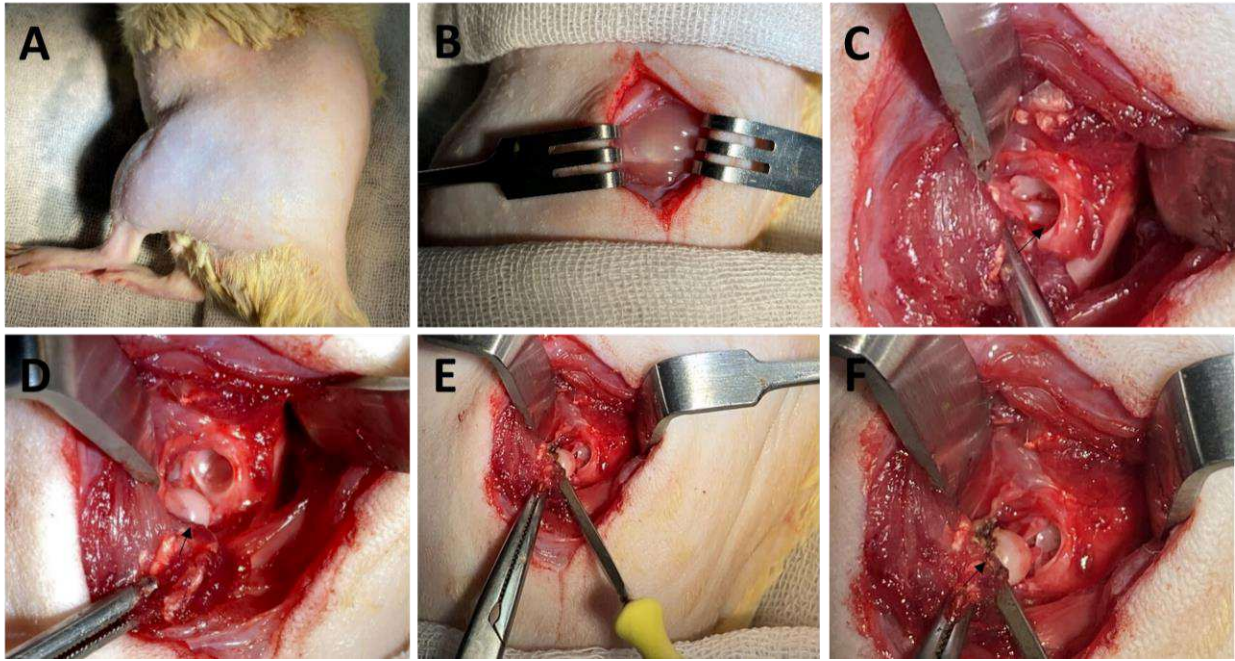


Figure 1. Intraoperative images of rat left femoral head; The skin over the greater trochanter was aseptically prepared (A); Skin incision and exposure (B); Dislocation from the acetabulum(C); Cutting of Ligamentum Teres (D); Deprivation of the blood vessel around the femoral neck circumferentially by using bipolar electrocoagulation (E, F).

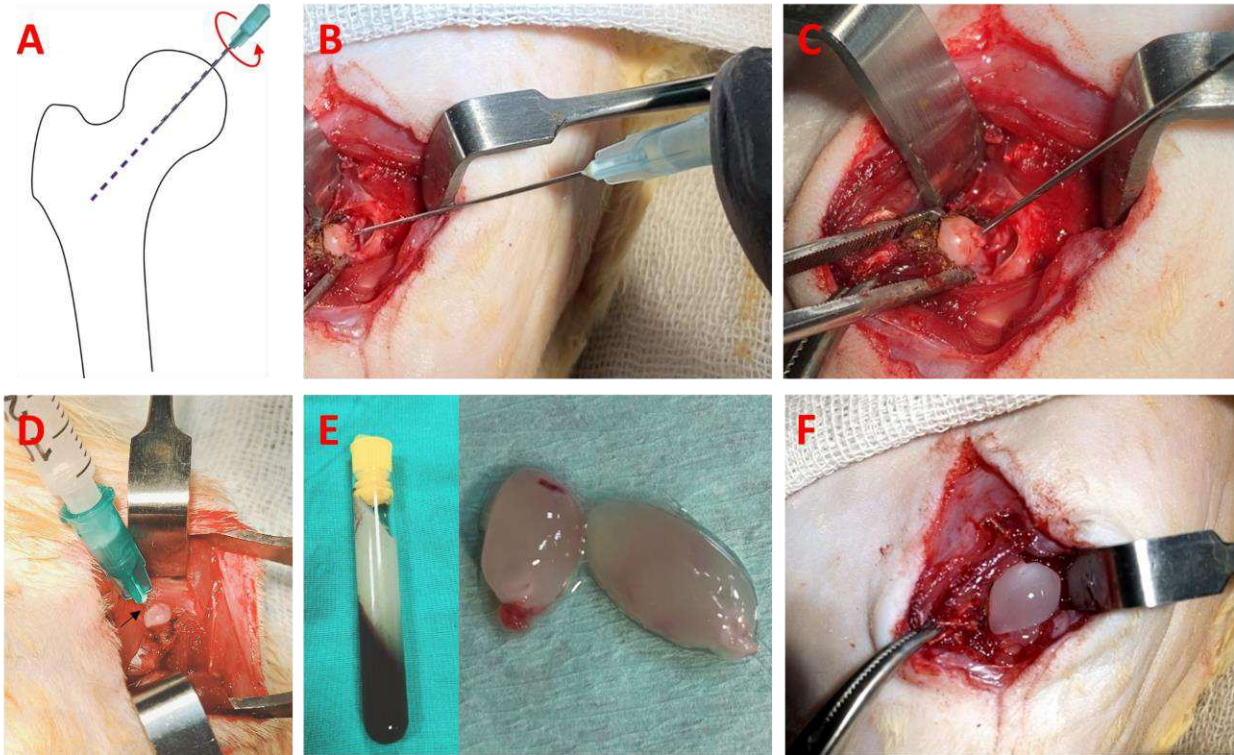


Figure 2. Diagram of creating CD tunnel in a rotating manner starting from the insertion point of the Ligamentum Teres through the head and the neck (A); Creating a decompression tunnel with a 21-gauge needle (B, C); Intraosseous injection of Nanoparticles (AgNPs, HA/AgNPs) with a 28 G needle (D); Preparation of PRF gel (E); Sealing of the tunnel with PRF gel (F).

Table 1: Steinberg staging system [29]

Stage	Findings
0	No symptoms Normal radiograph MRI non-specific
1	Mild pain in the affected hip, or pain with internal rotation Normal radiograph MRI diagnostic
2	Worsening or persistent pain Increased sclerosis or cysts in the femoral head
3	Subchondral collapse (crescent sign)
4	Flattening of the femoral head Normal joint space
5	Narrowing of the joint space with/without femoral head involvement
6	Advanced degenerative changes

Table 2. Comparison between the different studied groups according to nociceptive threshold (g). Data was expressed using Mean $\pm$ SD and significance was tested with two-way ANOVA test followed by Tukey multiple comparison; Means in the same Row with any small common letter ^(a-b) are not significant within group; Means in the same Column with any capital common letter ^(A-C) are not significant between different groups, significant at $p \leq 0.05$.

Nociceptive threshold	2weeks	4weeks	8weeks
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C-ve	22.33 ^{aA} ± 1.86	21.96 ^{aA} ± 1.63	23.83 ^{aA} ± 1.17
AVN	13.33 ^{aC} ± 1.86	11.33 ^{aC} ± 1.97	6.50 ^{bD} ± 2.07
CD	14.0 ^{aC} ± 2.37	12.50 ^{aC} ± 1.87	8.0 ^{bD} ± 3.10
CD-PRF-AgNPs	15.17 ^{aBC} ± 1.94	12.83 ^{aC} ± 1.72	11.67 ^{aC} ± 3.39
CD-PRF-HA/AgNPs	17.67 ^{aB} ± 1.75	16.50 ^{aB} ± 2.26	16.50 ^{aB} ± 3.15

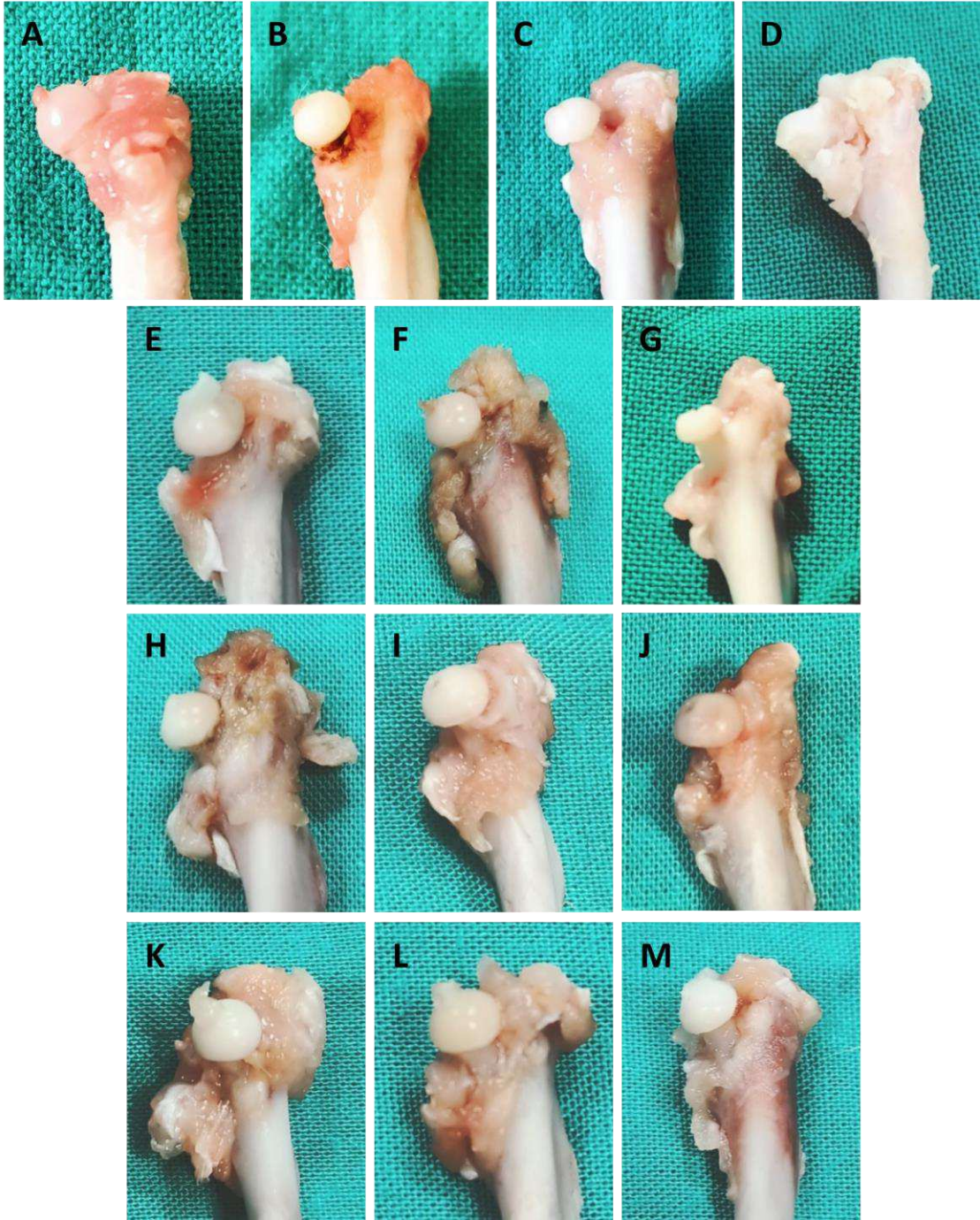


Figure 3. Gross morphological observations of AVN femoral head of the rat exhibited reservation of the sphericity of femoral head in 2 weeks (B); Complete flattening with shortening of femoral neck at 4 weeks (C); Notable defect with complete loss of the articular surface and visible femoral neck resorption (D) at 8 weeks in The AVN group ; Spherical, regular and glistering surface with no fibrillation or erosions (A) in C-ve group; Preservation of the femoral head sphericity at 2,4 weeks (E,F); Clear bone deformity and flattening of the femoral head (G) at 8 weeks in CD group; Grossly evident of preservation of the femoral head sphericity with no fibrillation or erosions at articular surface at 2 and 4 weeks (H, I); The gross observation of femoral head showed slightly loss of its sphericity at 8 weeks (J) in CD-PRF-AgNPs group; Complete restoration of the normal appearance of femoral head with smooth and regular surface covered by hyaline cartilage without any noticeable bony defect at 2,4,8 weeks (K,L,M) in CD-PRF-HA/AgNPs group.

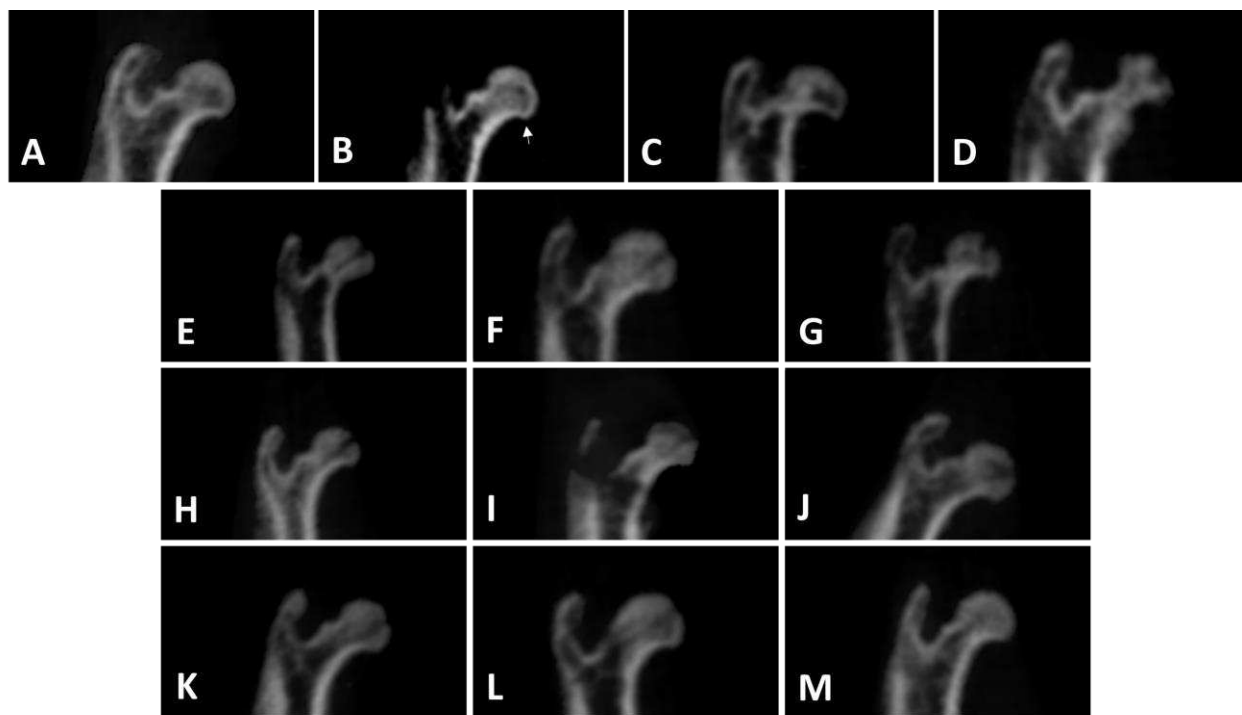


Figure 4. Cone beam computed tomography images of femoral head in the hip of AVN rat exhibited reservation of its sphericity and represented by subchondral collapse (crescent sign) with a small amount of high shadow in 2 weeks (white arrow; B); Loss of its sphericity with evident of radiographical changes represented by irregular hemispherical shape with uneven density as dark shadow and exhibited complete flattening of the femoral head with shortening of femoral neck at 4 weeks (C); Advanced degenerative changes with notable deformity with visible femoral head resorption and complete loss of the articular surface at 8 weeks (D); Spherical, regular shape, and normal morphology with uniform density and no evident of radiographical changes in C-ve group (A); Preservation of the femoral head sphericity with no obliteration of the tunnel at 2,4 weeks (E,F), subchondral collapse with visible femoral neck resorption, partial obliteration of the CD tunnel and lost their sphericity at 8 weeks in CD group (G); The sphericity of the femoral head were partially preserved with presence of tunnel at 2 weeks (H), partial obliteration of CD tunnel at

4, 8weeks (I, J) in CD-PRF-AgNPs group; Relatively reserved sphericity of femoral head with partial obliteration of the tunnel at 2 weeks (K) and 4weeks (L),restored femoral head shape with complete obliteration of the tunnel at 8weeks (M) in CD-PRF-HA/AgNPs group.

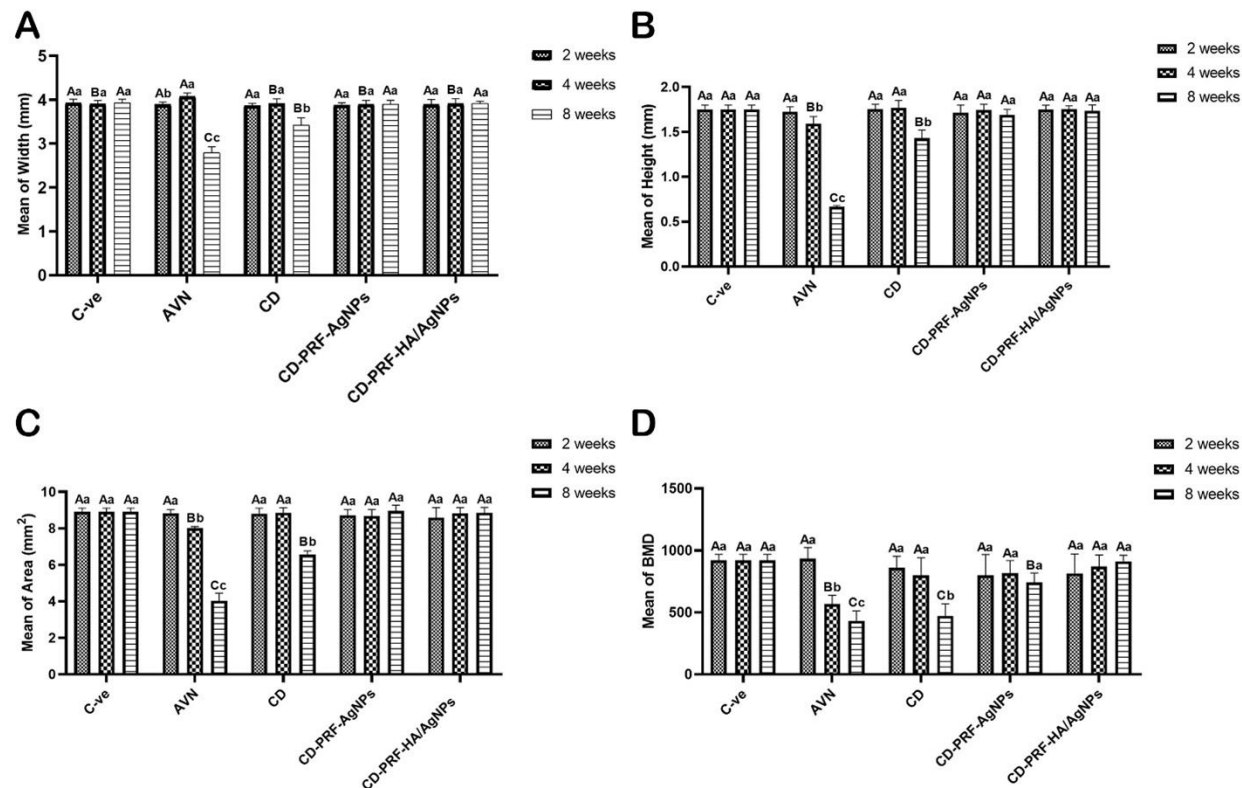


Figure 5. Bar graph representing the mean of width of femoral head (mm) (A); the mean of height of femoral head (mm) (B); the mean of area of femoral head (mm) (C); the mean of BMD of femoral head (D). Each subgroup (n=8). Data was expressed using Mean $\pm$ SD and significance was tested with two-way ANOVA test followed by Tukey multiple comparison; Means in the same row with any small common letter (a-b) are not significant within group; Means in the same column with any capital common letter (A-C) are not significant between different groups; Significant at $p \leq 0.05$.

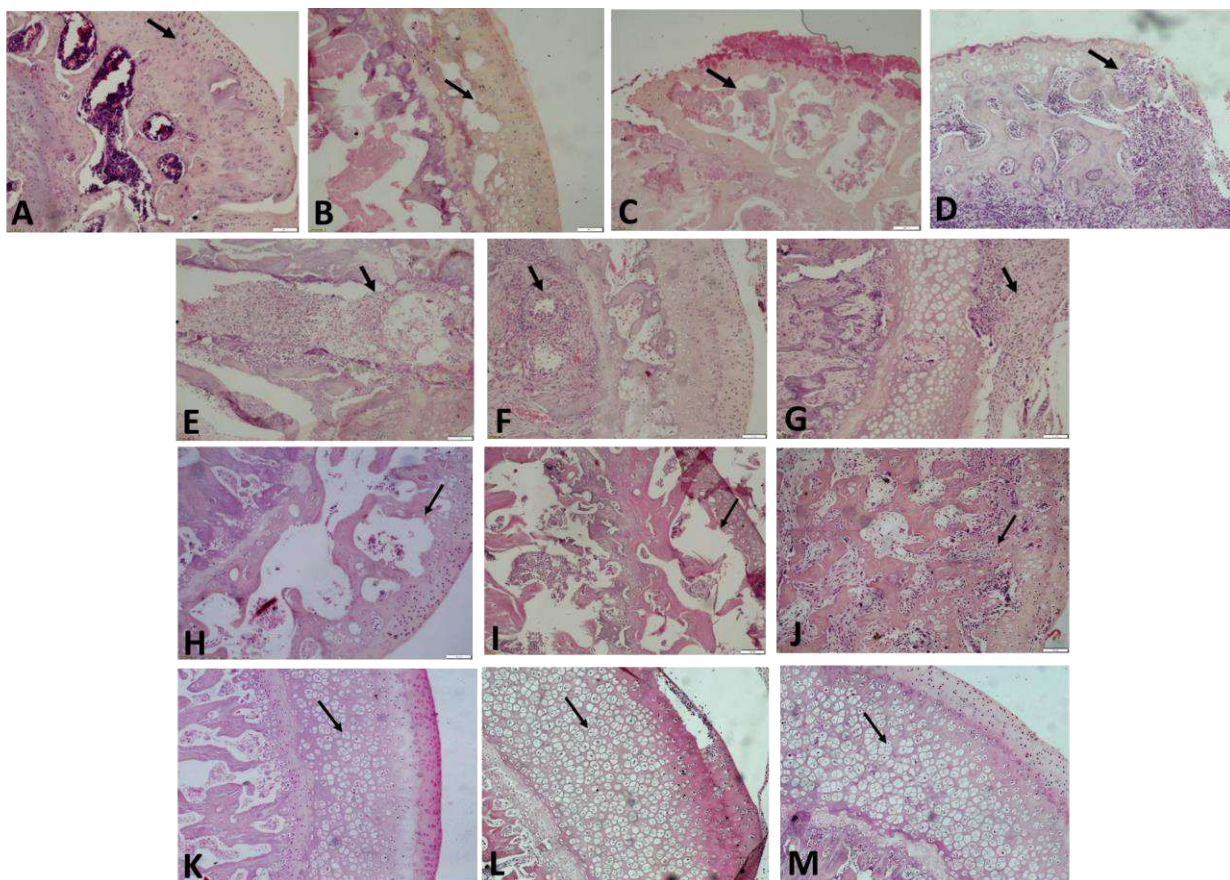


Figure 6. Histological view of femoral heads at 2, 4, 8 weeks after surgery. HE staining the AVN group at week 2,4,8 (black arrow; B,C,D) respectively (HE, 100x) ;The control group A (HE, 100x); The CD group at 2,4,8 weeks (black arrow ;E,F,G) (HE, 100x); The CD-PRF-AgNPs group at 2,4,8 weeks (black arrow; H,I,J) (HE, 100x); The CD-PRF-HA/AgNPs group at 2,4,8 weeks (black arrow; K,L,M) (HE, 100x).

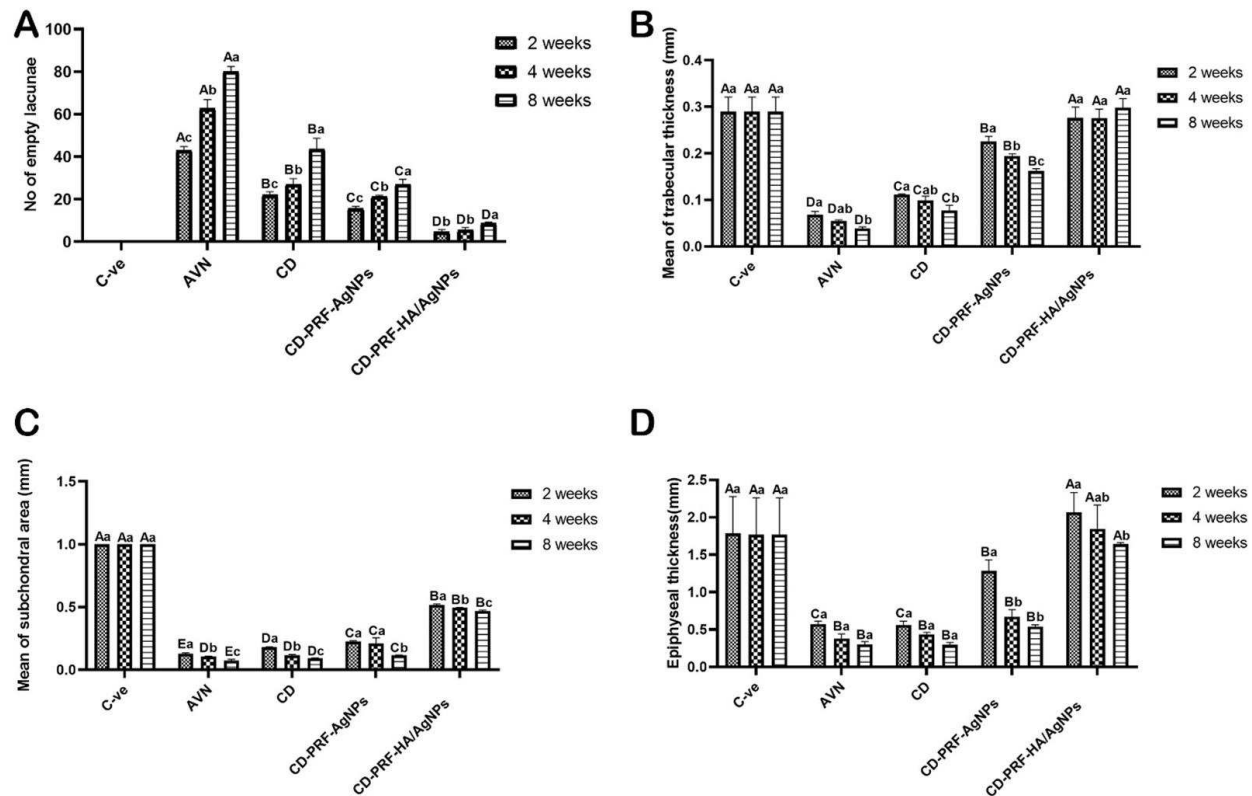


Figure 7. Bar graph representing the mean of number of empty lacunae of femoral head /1.8cm (A); the mean of trabecular thickness of femoral head (mm) (B); the mean of subchondral area of femoral head (mm) (C); the mean of epiphyseal thickness of femoral head (D). Each subgroup (n=8). Data was expressed using Mean $\pm$ SD and significance was tested with two-way ANOVA test followed by Tukey multiple comparison; Means in the same row with any small Common letter (^{a-b}) are not significant within group; Means in the same column with any capital common letter (^{A-C}) are not significant between different groups; Significant at $p \leq 0.05$.

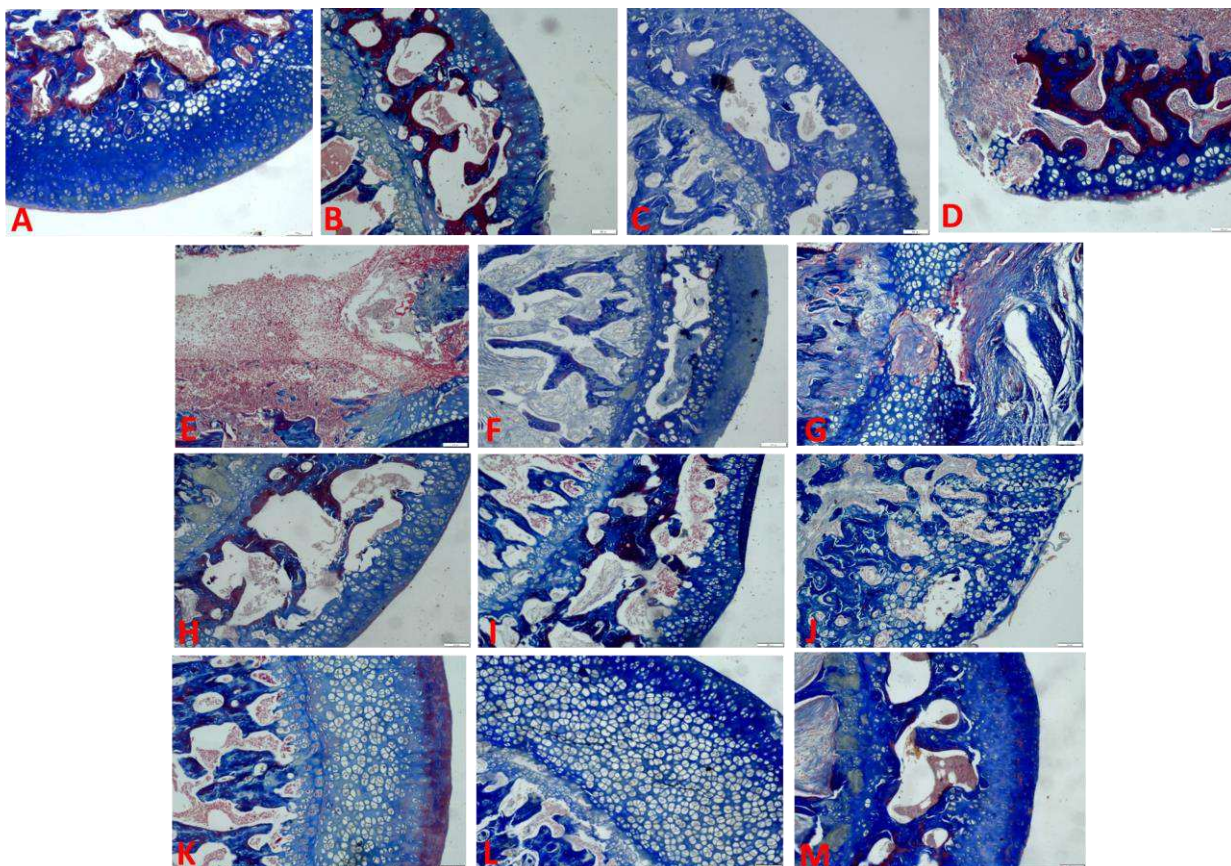
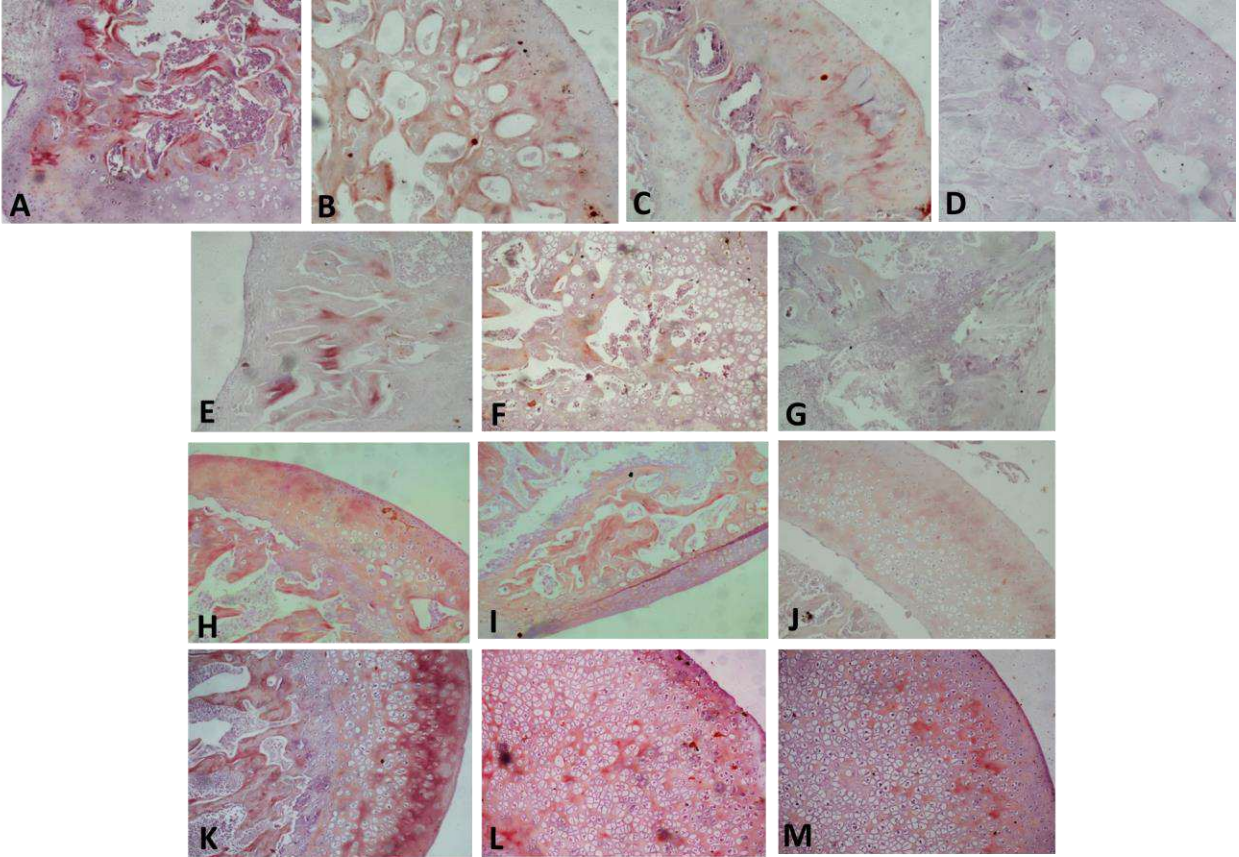


Figure 8. Histological view of femoral heads at 2, 4, 8 weeks after surgery. Masson trichrome staining the AVN group at week 2,4,8 B,C,D respectively (Masson trichrome, 100x) ;The control group A (Masson trichrome, 100x); The CD group at 2,4,8 weeks E,F,G (Masson trichrome, 100x); The CD-PRF-AgNPs group at 2,4,8 weeks H,I,J ((Masson trichrome, 100x); The CD-PRF-HA/AgNPs group at 2,4,8 weeks K,L,M (Masson trichrome, 100x).



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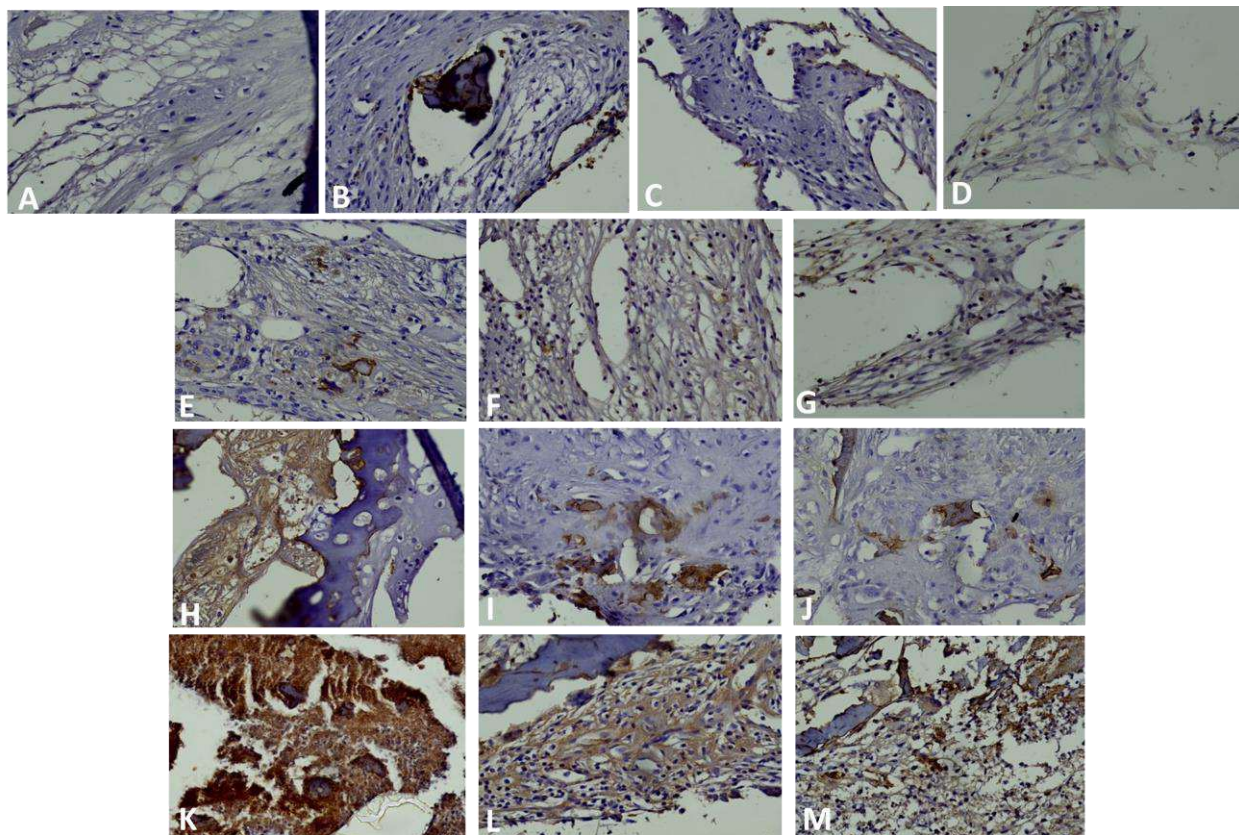
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Figure 9. Histological view of femoral heads at 2, 4, 8 weeks after surgery. Alizarin red s staining the AVN group at week 2,4,8 B,C,D respectively (Alizarin red s, 100x) ;The control group A (Alizarin red s, 100x); The CD group at 2,4,8 weeks E,F,G (Alizarin red s, 100x); The CD-PRF-AgNPs group at 2,4,8 weeks H,I,J (Alizarin red s, 100x); The CD-PRF-HA/AgNPs group at 2,4,8 weeks K,L,M (Alizarin red s, 100x).



417

418 Figure 10. Immunohistochemical staining of OPN of femoral heads at 2, 4, 8 weeks after surgery. the AVN group at
 419 week 2,4,8 B,C,D respectively (IHC, DAB immunostaining, hematoxylin as counter stain, 400x) ;The control group
 420 A (IHC, DAB immunostaining, hematoxylin as counter stain, 400x); The CD group at 2,4,8 weeks E,F,G (IHC, DAB
 421 immunostaining, hematoxylin as counter stain, 400x); The CD-PRF-AgNPs group at 2,4,8 weeks H,I,J (IHC, DAB
 422 immunostaining, hematoxylin as counter stain, 400x); The CD-PRF-HA/AgNPs group at 2,4,8 weeks K,L,M (IHC,
 423 DAB immunostaining, hematoxylin as counter stain, 400x).
 424

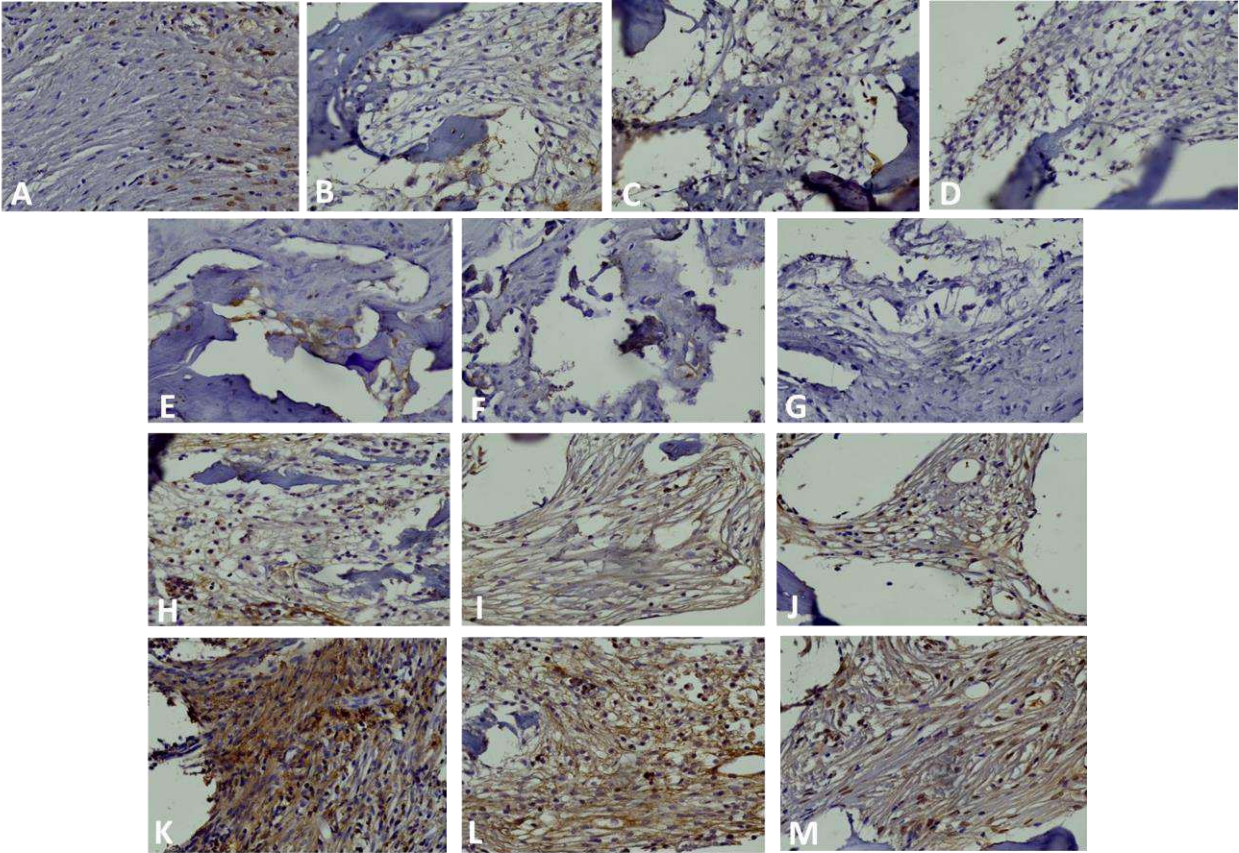


Figure 11. Immunohistochemical staining of CD34 of femoral heads at 2, 4, 8 weeks after surgery. the AVN group at week 2,4,8 B,C,D respectively (IHC, DAB immunostaining, hematoxylin as counter stain, 400x) ;The C-ve group A (IHC, DAB immunostaining, hematoxylin as counter stain, 400x); The CD group at 2,4,8 weeks E,F,G (IHC, DAB immunostaining, hematoxylin as counter stain, 400x); The CD-PRF-AgNPs group at 2,4,8 weeks H,I,J (IHC, DAB immunostaining, hematoxylin as counter stain, 400x); The CD-PRF-HA/AgNPs group at 2,4,8 weeks K,L,M (IHC, DAB immunostaining, hematoxylin as counter stain, 400x).

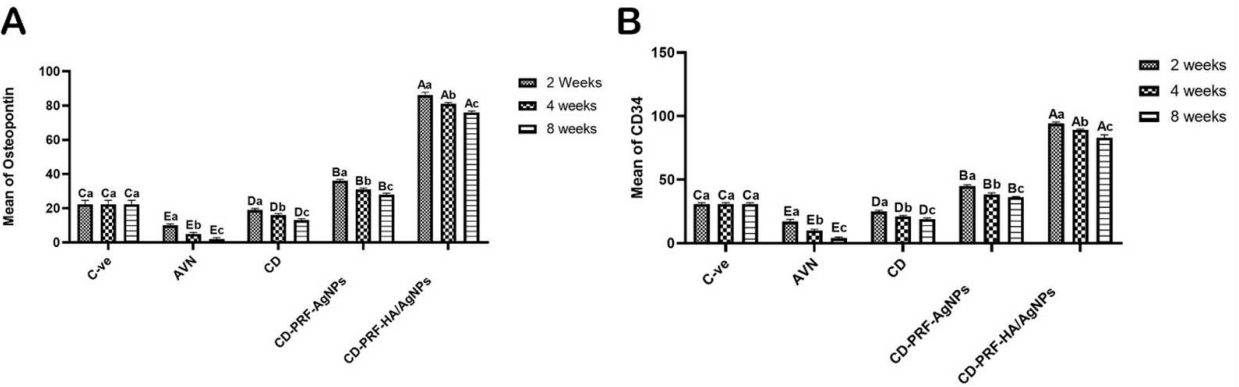


Figure 12. Bar graph representing the mean of OPN expression in the femoral head (A); Bar graph representing the mean of CD34 expression (B). Each subgroup (n=8). Data was expressed using Mean $\pm$ SD and significance was tested with two-way ANOVA test followed by Tukey multiple comparison; Means in the same row with any small

Common letter ^(a-b) are not significant within group; Means in the same column with any capital common letter ^(A-C) are not significant between different groups; Significant at $p \leq 0.05$.

4. DISCUSSION

Femoral head AVN is a form of osteonecrosis caused by disruption of the femoral head's blood supply, which can be caused by both traumatic and non-traumatic factors and results in cell death [35]. Ineffective repair of the osteoid tissue leads to joint collapse until osteoarthritis (OA) because it is physically and functionally cannot replace original bone. Common treatment for end-stage osteonecrosis is THA [36, 37]. So, the goal of AVN therapy is to prevent the development of femoral head collapse until complete deformity and preservation of joint function and integrity.

The present study demonstrated that the pure CD group showed superior outcomes compared to AVN group, this may be attributed to CD reduce intramedullary pressure in the necrotic area and promote revascularization thus delaying femoral head collapse in AVN [38]. However, it exhibited poor osteogenic and angiogenic activity with obvious degenerative changes in the femoral head over 8 weeks compared to the other treated groups based on macroscopic, radiographic and histological findings. This may be due to the lack of mechanical support of the necrotic area after decompression, the collapse of the bearing surface may be accelerated. In addition, the issues of angiogenesis, bone regeneration, and articular surface restoration in the necrotic area are not addressed by this approach [39]. In line with our findings, Peled et al. (2013) showed that CD, especially when combined with alendronate, improved femoral head preservation in an ischemic femoral head [20]. Similarly, Basal et al. (2018) demonstrated that combining CD with biological stimulation, such as epidermal growth factor, promoted angiogenesis and osteogenesis, revealing the potential of adjunctive therapies to augment the benefits of CD [40].

To reduce intramedullary pressure in the necrotic area and promote revascularization, bone formation, and remodeling by causing local bone injury, clinicians have been attempting to combine CD with autografts, vascular implants, and metallic implants [39].

PRF, a second generation platelet concentrate, is a strong framework rich in platelets, fibrin; leukocytes, circulating stem cells, healing and pro-inflammatory cytokines and growth factors which provides the continuous release of vital growth factors such as fibroblast growth factors (FGFs), vascular endothelial growth factors (VEGFs), transforming growth factors (TGFs) and

platelet-derived growth factors (PDGFs) that can stimulate vessel formation, cell differentiation and decrease the occurrence of inflammation [41, 42].

The current study showed that both CD-PRF-AgNPs and CD-PRF-HA/AgNPs groups significantly improved all macroscopic, radiographic, and histological parameters relative to the CD group alone, confirming a synergistic effect of PRF in improving the properties of graft materials when combined with it as mentioned previously by Mohammed et al.(2021) and Noronha Oliveira et al. (2023) who support its synergistic effect, as the sustained release of growth factors facilitated bone regeneration .This may be due to PRF providing a slow, steady, and sustained release of concentrated cytokines and growth factors, supporting cell proliferation, migration, and differentiation as well as enhancing healing at the ischemic site by encouraging local angiogenesis [43, 44].

The main reason for implant failure in orthopedic surgery is infection. To reduce this problem, scaffolds have been linked to antimicrobial agents including AgNPs and antibiotics. It has been demonstrated that adding AgNPs with antibacterial qualities and/or altering the surface of bone implants with AgNPs can reduce infections and enhance the healing of bone fractures [14].

The current investigation indicated that treating AVN femoral heads with PRF-AgNPs composite preserved the sphericity of the femoral head while exhibiting a considerable improvement in radiographic and histological markers compared to the AVN and CD groups, this result came in agreement with Senthil and Cakir (2024) who noted that AgNPs when combined with curcumin on demineralized bone matrix, encouraged nano-apatite growth, improved implant stability, and supported cell growth, demonstrating their benefits in bone regeneration [45]. This could be because AgNPs incorporated angiogenic materials have excellent biological properties on cells that influence MSC cell proliferation and differentiation and enhance the materials' performance with growth factors for bone healing and neovascularization [46].

However, based on CBCT and histopathological analysis, the CD-PRF-AgNPs showed an incompletely obliterated CD hole with no noticeable change in calcification or neovascularization and displayed deterioration in femoral head's architecture compared to CD-PRF-HA/AgNPs group. This may be attributed to although AgNPs exhibiting effective antibacterial properties and promoting bone regeneration, they lack the complete biological activity needed for bone

regeneration such as osteogenesis, angiogenesis and bone matrix deposition. So, AgNPs alone cannot confirm complete bone healing and require combination with other regenerative agents, such as growth factors or biomaterials, to accomplish ideal outcomes [14, 47]. Therefore, the current study showed that even while growth factor therapies are important for enhancing the healing process, they did not provide the bone enough time to repair by providing initial biomechanical support for the collapsed head because of recurrent injury.

The present study demonstrated that the combination of decompression of the medullary center and implantation of PRF gel with HA/AgNPs enhanced the formation of new osteoid tissue, mineralization and vascularization of the necrotic femoral head, as well it enhanced bone fill percentage with radiographic evidence of full CD hole repair and recorded an increase in BMD with totally preserved femoral head sphericity. It could be attributed to the nanostructure of bone tissue making nanonization of HA-based biocomposites one of the most promising challenges in bioceramics. Nano hydroxyapatite, which constitutes about 60–70% of bone, closely mimics cancellous bone microarchitecture. It has also garnered a lot of attention as an orthopedic biomaterial due to its exceptional osteoconductivity, bioactivity, and biocompatibility [48, 49].

Fouad-Elhady et al. (2020) demonstrated that HA-based interventions modulated bone turnover and improved bone stability in an experimental osteoporosis rat model. This finding also supports the point that HA's significant resemblance to bone matrix and capacity to create chemical interactions with the host tissue give it a better advantage in clinical applications than most other bone substitutes, such as allografts or metallic implants. Thus, HA/AgNPs composites not only provide the antibacterial action of AgNPs but also enhance osteoconduction and integration with osteoid tissue, allowing them more efficient in promoting regeneration and mechanical stability of the femoral head than AgNPs alone [13].

The femoral head's AVN is an ischemia event that frequently results in severe pain, joint dysfunction, and, in later stages, joint collapse. Successful therapy focus on regenerating the femoral head, maintaining joint function and alleviating inflammation and pain to an ideal state that resembles the normal condition [50]. This study evaluated pain response by focusing on the changes of nociceptive threshold to mechanical hyperalgesia using an algometer. This is a common technique used in osteoarthritis (OA) models, which provides objective records of mechanical nociceptive thresholds through application of pressure. Comparable methods have

been used to assess joint pain and treatment effects in OA. With the common underlying pathologic points of AVN and OA such as subchondral bone damage, and inflammation [51], such a method is not only practical but also an innovative one in the assessment of experimental osteonecrosis models.

The study found that the CD-PRF-HA/AgNPs group exhibited the best improvement in mechanical hyperalgesia nociceptive threshold and body weight at each time point indicating improved pain sensation and enhanced joint function. On the other hand, CD and AVN groups exhibited significant decreases in these measures, attributing that to severe destruction of bone architecture, reduced potential for bone regeneration leading to loss of the joint function and severe pain sensation. This aligns with Andrei et al. (2023), who used an algometer to determine that cetirizine and levetiracetam improved pain thresholds in an OA model, revealing their analgesic effect [52] .

AVN of the femoral head has multifactorial pathophysiology, but exactly, it remains unclear. However, the gross lesion remains constant [53]. The typical gross observation of the AVN group showed a complete loss of its articular surface and femoral neck resorption at 8 weeks. Although the femoral head in the CD group was preserved at 2 and 4 weeks but had significantly flattened at 8 weeks, this could be attributed to the lack of the angiogenic and osteogenic factors that encourage the regeneration of the necrotic area [54]. Conversely, at each time point, the CD-PRF-HA/AgNPs group restored a smooth, regular articular surface with reserved femoral head sphericity, resembling normal morphology and reflecting the positive effect of PRF-HA/AgNPs composite in preventing AVN progression. Also, Chen et al. (2018) and Xu et al. (2023) noted these degenerative changes in macroscopical observations in their studies in AVN rat model [55, 56].

MRI is the radiographical method usually employed for identifying and assessing the healing progress of AVN [57].Based on our knowledge till now, there was no researches about the application of CBCT as a confirmatory method in AVN subsequent evaluation, but it is frequently used in dental medicine in diagnosis and management of jaw osteonecrosis [58].A preclinical trial for CBCT as new radiographical method was performed to evaluate the healing and preservation of AVN animal model .

This study displayed the potential of CBCT to identify degenerative alterations such as subchondral sclerosis and collapse with changes in BMD (these changes are hallmarks of AVN). CBCT is also used to evaluate the healing progress of the ischemic femoral head. Likewise, CBCT was previously employed by Zalama et al. (2022) in assessment of the repair of the critical bone defects in rabbits [19].

CBCT demonstrates limited sensitivity in identifying early stage of AVN, it is suited for evaluating bone structure rather than marrow abnormalities, and does not afford physiological or metabolic details. The study overcomes CBCT limitations using microscopic examinations which allow us to identify AVN in earlier stage by detecting deteriorations of the osteoid architecture, empty lacunae, and vascular changes revealing early ischemic injury which can't be detected by CBCT alone [2].

According to CBCT measures, the AVN and CD groups showed subchondral collapse with loss of sphericity and flattening of the head at 4 weeks, as well as some degree of deformity which was verified by a notable reduction in the femoral head's height, width and area with obvious femoral neck resorption. They also showed a significant decrease in the femoral head's BMD over 8 weeks. CD-PRF-AgNPs also demonstrated a significant decrease in BMD with partial obliteration of the CD hole at 8 weeks. Conversely, the CD-PRF-HA/AgNPs group displayed a significant restoration of these measurements resembling C-ve group, this could be explained by the addition of HA nanoparticles to AgNPs has a beneficial effect on femoral head regeneration and protects it from collapsing. These results were consistent with other findings [15, 27, 28] who analyzed these radiological measurements for indicating confirmative structural preservation or degeneration among the different groups in osteonecrosis model.

The microscopical examination of AVN group demonstrated that the architecture of the ischemic femoral head deteriorated which was previously noticed in rat model of osteonecrosis of femoral head by Lv et al., (2022), and Ma et al., (2022) [59, 60]. On the contrary, The CD-PRF-HA/AgNPs group demonstrated by notable histological improvement with restored normal bone and osteoid matrix compared to both CD and CD-PRF-AgNPs groups which had significant deterioration and were unable to prevent bone turnover. This result may be ascribed to its nano-size and shape which resemble closely those of HA crystals in the natural bone mimicking the environment in which the

cells grow and provide a place for angiogenesis and differentiation which is compatible with the findings of Fouad-Elhady et al., (2020) who used HA/AgNPs as a therapeutic for management of osteoporotic rats [13].

The findings of clinical and CBCT analysis were in line with the histopathological measurements, The group receiving PRF-HA/AgNPs composite demonstrated a significant improvement in histopathological measurements compared to the other treated groups. This may be attributed to HA/AgNPs and PRF gel suppressing the apoptosis of osteoblasts and osteocytes. Conversely, there was a significant decrease in trabecular and epiphyseal thickness and subchondral area with an abundance of empty osteocyte lacunae, the hallmark of osteonecrosis, which increased sharply over time indicating severe pathological deteriorations in AVN group, which is consistent with Basal et al.(2018) who assessed the histopathological deterioration in AVN rat model by analysis of these measurements (trabecular and epiphysis thinness and decreased subchondral area. Also, Ma et al., (2022) measured the percentage of empty lacunae which increased sharply over time, indicating severe pathological deteriorations in AVN model [40, 61].

Masson trichrome staining displayed osteoid matrix loss that increased over time, along with extensive regions of bone resorption in AVN group. However, compared to other treated groups, the CD-PRF-HA/AgNPs group displayed strong bluish staining, indicating restoration of osteoid matrix that was close to normal architecture as showed in previous studies using Masson Trichrome stain in histopathological examination of AVN model [27, 28].

The degree of mineralization was evaluated using Alizarin red S staining. During the bone matrix deposition process, Alizarin red S staining reacted with calcium to precipitate calcified nodules [62]. This study displayed decreased calcified nodule areas with depletion in staining intensity in the AVN ,CD and CD-PRF-AgNPs groups compared to the C-ve group. In contrast, because of better bone matrix deposition, the PRF-HA/AgNPs group displayed the strongest staining intensity. This aligns with previous studies [33, 63]. This improvement may be attributed to the superior effect of HA/AgNPs which convert mechanical stimuli into biochemical signals and induce bone matrix mineralization, is responsible for this improvement. These nanoparticles also encourage apatite nucleation and mineralization through osteoblast activity.

The mineralization and angiogenesis processes of the bone extracellular matrix, which are essential for bone growth, healing, and structural integrity, are mediated by non-collagenous proteins such as Osteocalcin and OPN, in this investigation, the OPN levels in treated groups were significantly greater than the AVN group. However, the CD-PRF-HA/AgNPs group recorded the highest levels compared to other treated groups. This could be explained by the enhancement of bone mineralization and matrix deposition by nano-hydroxyapatite is responsible for this rise. After an ischemic insult, elevated OPN levels show that efforts are being made to rebuild bone tissue through mineralization, cell-matrix organization, and angiogenic signaling, especially during the ischemic phase, which enhances the creation of osteoid tissue and osteoblast differentiation, These findings are consistent with research by Campos Pessoa et al. (2021) who reported that intense expression of OPN representing enhanced early bone regeneration in a rat model of ischemic osteonecrosis in treated group [64]. On the other hand, another study demonstrated that the OPN levels of the CD and treated groups did not significantly vary [15].

Any therapeutic agent that targets AVN in the femoral head must restore bone vascularity, which is an absolute criterion in the osteoinduction process. The present experiment used CD34 staining to evaluate angiogenesis which showed that the scaffold groups had higher levels of CD34 expression than AVN and CD groups. This result matched previous study by Dailiana et al. (2018) who found that VEGF treatment increased CD34 expression in femoral head osteonecrosis in dogs reflecting enhanced neovascularization [65]. This could be because the addition of nanomaterial along with PRF gel via CD tunnel may optimize the CD surgery by enhancing angiogenesis of the ischemic femoral head.

5. Conclusion:

The present study demonstrated that HA/AgNPs combined with PRF gel administered locally in ischemic femoral head can optimize CD interference showing considerable therapeutic potential resulting in restoration of osteoid tissue architecture and BMD, enhancing osteogenesis and angiogenesis, preventing joint collapse and promoting bone regeneration in the early stages of AVN.

Abbreviation

AVN: Avascular necrosis

641 AgNPs: Silver Nanoparticles
642 HA/AgNPs: Silver hydroxyapatite nanoparticles
643 HANPs: Hydroxyapatite nanoparticles
644 PRF: Platelets rich fibrin
645 CD: Core decompression
646 CBCT: Cone beam computed tomography
647 OPN: Osteopontin
648 CD34: Cluster of differentiation 34
649 THA: Total hip arthroplasty
650 CT: Computed tomography
651 IP: Intraperitoneal
652 HA: Hydroxyapatite crystals
653 FGFs: Fibroblast growth factors
654 VEGFs: Vascular endothelial growth factors
655 TGFs: Transforming growth factors
656 PDGFs: Platelet-derived growth factors
657 OA: Osteoarthritis

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661 **Availability of data and materials**

662 The datasets used and/or analyzed during the current study are available from the corresponding
663 author upon reasonable request.

664 **Ethics approval and consent to participate:**

665 The present study was approved following the ethics of the Mansoura University Animal Care and
666 Use Committee (Ph.D/105) which met the ARRIVE guidelines.

667 ‘Clinical trial number: not applicable.’

668 **Consent for publication**

669 Not applicable

670 **Declaration of competing interests**

671 There are no conflicts of interest to declare. This manuscript has not been published elsewhere and
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677 **Authors' contributions**

678 AA: Study design, experimental operation, statistical analysis, data interpretation, manuscript
679 writing, and editing.

680 AR: Study design, data collection, and manuscript writing.

681 MM: Surgical procedures, Interpretation of CT findings.

682 MH: Histopathological examination and interpretation

683 MM: Data collection, and manuscript writing.

684

685 EM: Study design and manuscript editing.

686 AZ: Study design, manuscript editing

687 GK: Study design, manuscript editing, and final revision.

688 All authors have read and approved the final submitted manuscript

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