



Bone marrow concentration in combination with hyaluronan and fibrin for the treatment of primary and non-primary in orthopaedic cases of osteochondral defects of the ankle

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ABSTRACT

This research examined the effects of varying amounts of fibrinogen (the fibrin precursor) and HA-MA on mechanical strength, BMSC proliferation, and chondrogenesis potential in vitro. In order to start a culture, fibrinogen, aprotinin, doxycycline, and HA-MA were well mixed in 200 L of AMEM containing 1% (w/v) photoinitiator. In order to produce a fibrin gel, thrombin was used at 1, 3, 5, and 7 days after implantation, live/dead staining and a metabolic-activity test were used to examine the viability and proliferation of BMSCs inside fibrin/HA-MA. The cell lysis solution from the Real Time ready Cell Lysis Kit was added to each gel. Both primer and probe mixes, as well as DNA polymerase, deoxyribonucleotide triphosphates, an activator, and an enhancer, were mixed before the lysis process began to assess mRNA expression. The mechanical strength of fibrin hydrogels was shown to be proportional to the quantity of HA-MA utilised in the reinforcement. Quantitative polymerase chain reaction demonstrated a reduction in the expression of collagen type I alpha 1 mRNA in BMSCs after they were treated in a fibrin/HA-MA hydrogel. Using fibrin/HA-MA hydrogel as a delivery technique for bone marrow stem cells may induce BMSC differentiation into chondrocytes and perhaps aid in articular cartilage repair for OA therapy (BMSCs). We concluded that the utilisation of bone marrow concentration in conjunction with a combination of fibrin and Hyaluronan treatment is safe for patients suffering from OCD of the ankle and is well tolerated by these patients as both a primary treatment and a non-primary treatment option.

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Introduction

It is estimated that 27,000 persons inside the borders of the United States of America suffer from ankle sprains on a daily basis (1). Up to fifty percent of all instances of acute ankle sprains and fractures have a chance of causing injury to the osteochondral region of the ankle (OCL). Since cartilage does not contain any blood arteries, it has a weak propensity to heal, which means that these flaws have the potential to evolve into osteoarthritis. This condition may be painful and debilitating. The ensuing plodding and persistent march towards rising size and severity may finally develop in osteoarthritis or degenerative arthrosis if not treated. This may occur if the problem is not addressed in a timely manner (2). An osteochondral defect is a condition that occurs when there is a problem with both the articular cartilage and the subchondral bone that lies below it (OCD). This is a comprehensive explanation of the ailment (3). The majority of the time, this condition is brought on by a single traumatic incident or a succession of traumatic experiences, which finally leads to the fragment being either partly or entirely divorced from the whole.

Due to the irregularities, bearing weight causes a significant amount of pain in the ankle. You may have reduced function, a limited range of motion, stiffness, catching, locking, and edema. There is also a risk that you will have stiffness. Due to these symptoms, the person could find it

difficult to move about, do their job, or take part in sports. Injuries to the ankle, particularly sprains and fractures, are responsible for the great majority of congenital abnormalities that become apparent in young adults between the ages of 20 and 40 (4). The categorization of osteochondral lesions of the talus that Berndt and Harty devised in 1959 is the one that is most often used today. They came up with this classification. This categorization has four stages, and it is determined by the degree to which the osteochondral fragment has been displaced from its original position. Stage I indicates a tiny focal subchondral trabecular compression region, Stage II indicates a somewhat loose fragment, Stage III indicates a fragment that is loose but has not been displaced, and Stage IV indicates a fragment that is loose and has been displaced from its bed. Stage I indicates a tiny focal subchondral trabecular compression region.

Stage II indicates a fragment that is somewhat loose. Stage III indicates a fragment that is loose but has not been displaced. As a result of recent advancements in imaging techniques that are sensitive to cartilage, such as magnetic resonance imaging, magnetic resonance imaging (MRI), and computed tomography (CT), OCLs of the ankle are being found more frequently. This calls for a more extensive and in-depth investigation of therapeutic options that are already available (5).

The major limitation of computed tomography is that it is unable to provide information on the quality of the arti-

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cular cartilage (CT). Despite this, CT is the most important resource for examining bone changes connected with injury, measurement, and location (6). The information gained by MRI makes it possible to evaluate articular cartilage, as well as to ascertain whether or not subchondral inflammatory modifications are present and to ascertain the extent of the chondral lesion (7). As a direct consequence of this, it is regarded as the gold standard of diagnostic performance when it comes to OCLs. In 2007, Raikin and colleagues conducted a study to identify the anatomical region that was impacted the majority of the time. The lateral area was the second most common location where lesions were found to be found (8). On the other hand, lateral lesions have a propensity to be more symptomatic than medial lesions (8-9). In comparison to the central and lateral regions of the ankle joint, the medial and lateral centres of the joint have a greater baseline contact pressure than the central and medial sections of the joint (9).

Examples of traditional surgical procedures include debridement and removal of damaged cartilage or loose bodies, techniques of bone marrow stimulation, cell-based healing approaches, and the use of biological agents. These are the primary categories that various surgical operations may be categorised under. Excision is a method that includes cutting off the diseased region and then cleaning up the area once it has been removed. Excision is one of the surgical options available for mending damaged cartilage. In addition to OAT and chondrocyte implantation, other surgical procedures, such as MF and OAT and ACI, are available. Using platelet-rich plasma, bone marrow concentrate, mesenchymal stem cells, and fragmented juvenile articular cartilage are only some of the more modern approaches that have been developed (PJA) (10). An aspirate of bone marrow may include both mesenchymal stem cells and hematopoietic stem cells (11). It has been hypothesised that a concentration of bone marrow aspirates may help in the regeneration of tissue, thereby improving the quality of cartilage repair through an increase in aggrecan content as well as the level of tensile strength of the tissue. This is supported by the fact that bone marrow aspirate concentrates have been shown to have anti-cancer properties (12).

As a result of this, BMAC helps to create an environment that is potentially beneficial for the synthesis and repair of hyaline cartilage, while at the same time reducing the production of fibrocartilage (13). Hence, the purpose of this study is to evaluate the effect of osteochondral defects of the ankle on the treatment of primary and non-primary orthopaedic cases using concentrated bone marrow in conjunction with a mixture of hyaluronan and fibrin.

Materials and Methods

Materials

Human plasma fibrinogen, thrombin (800-1200 IU/mL), ethanol, CD44-fluorescein isothiocyanate, phosphate-buffered saline, trypsin, alpha minimal essential medium, bovine serum albumin, methacrylic anhydride (MA), hyaluronic acid (HA), sodium hydroxide. Quantikine ELISA kit for detecting TGF levels, Real Time Ready Cell Lysis Kit, RNA Master Hydrolysis Probes kit, aggrecan (ACAN), SOX9, collagen type 2 alpha 1, and collagen type I alpha 1 probes, and foetal bovine serum (FBS) and its constituent components. A live/dead analysis was per-

formed.

Equipment's

Hydrogel samples, Proton nuclear magnetic resonance spectroscopy, Spectrometer, field-emission scanning electron microscopy, Auto Sputter Coater, electron microscope, and a LightCyclerII 480 PCR platform.

Culture

Every piece of information on the donor of bone marrow-derived mesenchymal stem cells (BMSCs) was kept strictly secret. The buffy coat layer of bone marrow was extracted, and then it was plated in tissue culture flasks with DMEM that had been supplemented with 10% FBS and 5 g/mL doxycycline. The bone marrow was fractionated by centrifugation. The density of cell seeding was 1 x 10⁶ cells per square centimetre. After cleaning the media with phosphate-buffered saline, new media were used for the incubation process after every two days. At a temperature of 37 degrees Celsius, the cells were incubated with 5% CO₂ and 5% oxygen. After a week, the colony development was evaluated, and trypsin was used to harvest the cells. After counting the cells, they were replated in AMEM with 10% FBS and 5 g/mL doxycycline at a density of 6000 cells/cm². At a temperature of 37 degrees Celsius, flasks were moved to an environment containing 21% oxygen and 5% carbon dioxide. After repeating this process after every two days until roughly more than eighty percent of the cells had merged, the medium was switched.

Bone marrow-derived mesenchymal stem cells verification

There is evidence that CD44 functions as a phenotypic MSC marker as well. The Accuri C6 Flow Cytometer was used in order to evaluate the surface markers CD105-PE, CD73-PerCP, CD90-APC, CD44-FITC, and CD34-FITC. Centrifuged cells were washed in phosphate-buffered saline that contained 2% bovine serum albumin. The process was repeated with about 25,000 cells for each surface protein and another 25,000 for the four-color analysis. After adding the antibody surface markers along with the cells, the mixture was then treated for two hours in a solution containing 2% bovine serum albumin and phosphate-buffered saline. In preparation for examination by the flow cytometer, the cells were resuspended in PBS.

Modification of HA

HA sodium salt was suspended to a final concentration of 1% (w/v) in a diH₂O solution. After placing the solution in a flask with a spherical bottom, it was cooled to 5 degrees Celsius in a water bath while being stirred. The pH of the solution was kept constant at 10 by adding 5 N sodium hydroxide. Following a period of 24 hours, the reaction mixture was gradually added to an excess of ethanol in order to precipitate the product. After transferring the hyaluronic acid-methacrylicanhydride (HA-MA) to a round-bottom flask, it was then subjected to a vacuum drying process to eliminate any excess ethanol. After being dried, the sample was dialyzed for three days against diH₂O while receiving daily rehydration during the process.

Fibrin/HA-MA hydrogel preparation with BMSCs

In order to start a culture, fibrinogen, aprotinin, doxycycline, and HA-MA were well mixed together in 200 L

of AMEM containing 1% (w/v) photoinitiator. In order to produce a fibrin gel, thrombin was used. When the gelation process was finished, the gels were subjected to UV radiation at 350 nm for five minutes in order to cross-link the HA-MA. The samples of BMSCs were grown in AMEM with doxycycline at 37 degrees Celsius and 5% carbon dioxide. Just five minutes of exposure to UV radiation were allowed in order to prevent the light from having a damaging impact on the DNA and proteins contained inside the cells.

BMSC viability and proliferation

At 1, 3, 5, and 7 days after implantation, live/dead staining and a metabolic activity test were used to examine the viability and proliferation of BMSCs inside fibrin/HA-MA. After adding CalceinRed-Orange to a final concentration of 2 M, the mixture was allowed to incubate for 15 minutes before the results were seen. The vitality of BMSC was examined using fluorescence microscopy after the incubation period. Cultures were exposed in order to determine the rate of cell proliferation. A tenfold dilution of PrestoBlue® was added to the medium, and it was shaken orbitally for a period of two hours while incubating. This was done with regard to the total amount of gel and media. After removing the medium, the fluorescence level was determined using a microplate reader.

Testing and Field emission scanning electron microscopy

It was decided not to use BMSCs in the preparation of the hydrogels that had a volume of 1 mL. PBS was added so that the final gels could retain their complete hydration for a whole period of time. Compression tests using a load cell with a capacity of 5 N were carried out using the material testing system. The compression test was carried out at a strain rate of 2 millimetres per minute up to 80% strain. In preparation for mechanical testing, the modulus was determined to be 20% stretched while in the first linear zone. Fibrin gels with a concentration of 6 mg/mL fibrinogen were evaluated for emission scanning alongside gels containing 1 mg/mL HA-MA. After being lyophilized, the samples were transferred into a vacuum chamber to undergo sputter coating. After that, the specimen was placed in the field emission scanning electron microscope so that it could be imaged.

mRNA expression analysis

Platelet lysate was added to 6 mg/mL fibrinogen gels for 12 days to cultivate BMSCs with or without HA-MA (PL). While making PL, the same blood draw and donor were used. Following collection, PBS was used to wash the gels before they were centrifuged into a pellet. Both the aggrecan and COL1A1 probes, as well as the left and right primers, were combined per the manufacturer's instructions. The proper treatments were implemented on the LightCycler II 480 PCR platform. The cell lysis solution from the Real Time ready Cell Lysis Kit was added to each gel. Both primer and probe mixes, as well as DNA polymerase, deoxyribonucleotide triphosphates, an activator, and an enhancer, were mixed together before the lysis process began. The primers and probes were mixed together once their concentrations were optimised. This package comes with a template, PCR-grade water, and both master mixes.

Statistical analysis

Data were gathered and then submitted for statistical analysis. The one-way analysis of variance (ANOVA) was performed to obtain p-values, which were then utilised to assess whether or not there was statistical significance between all of the groups (p 0.05). Unpaired Student's t-Tests were used in order to establish whether or not a statistically significant difference existed (p-values were calculated to be less than 0.05).

Results

BMSC phenotype verification

After treating BMSCs with the fluorescently labelled antibodies (Fig 1) CD105-PE, CD73-PerCP, CD90APC, CD44-FITC, and CD34-FITC, the expression of phenotypical surface proteins was validated by flow cytometry.

The SSC quantifies the granularity of a cell, whereas the FSC measures its total size. intensities of fluorescence emitted by fluorescein isothiocyanate (FL1), phycoerythrin (PE), peridinin chlorophyll protein complex (PerCP), and fluorescein (FL4) channels (allophycocyanin, APC). Antibody fluorescent tags cause a change in fluorescence channel emission from positive markers. All individual tests for each marker came out successful. It was the unlabeled cell population that was utilised to draw the grid. Hence, it was shown that the BMSCs had the usual phenotypic required for chondrogenesis.

BMSC viability & proliferation

After 1, 3, 5, and 7 days of growth in various fibrin/HA-MA hydrogel formulations, BMSC activity was tested using a metabolic assay (Tables 1,2 Figures 2,3) There were no statistically significant changes seen between the fibrinogen groups at a concentration of 4 mg/mL and 6 mg/mL. Yet, the amount of HA-MA present in each group affected the activity of the cells. According to the results of a one-way analysis of variance (ANOVA), there was a statistically significant difference (p 0.05) between the two groups. In addition, we used the student's t-Test to determine the p values that differentiate each unique group pair. With the incorporation of HA-MA into the hydrogels, fibrinogen concentrations of 4 mg/mL and 6 mg/mL supported significant cell activity with HA-MA concentrations below 1 mg/mL. This was the case regardless of whether the fibrinogen was dissolved or not. Because of the pressures that were put on the gel matrix by the contraction of the

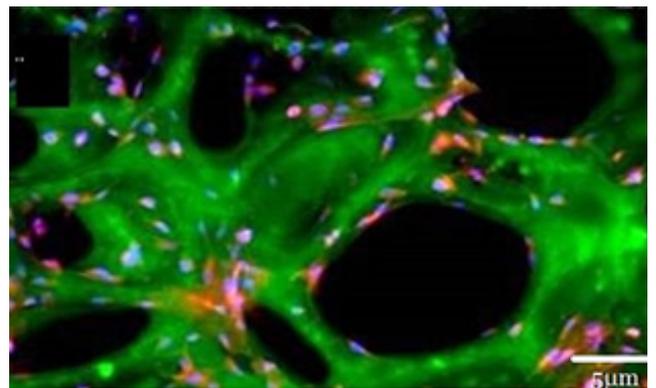


Figure 1. BMSCs with the fluorescently labelled antibodies.

Table 1. In vitro evaluation of BMSC metabolic activity in 4 mg/mL HA-MA-infused fibrin/HA-MA hydrogels.

Relative fluorescence's	HA-MA Concentration				P value
days	0.0	0.5	1	1.5	
1	290	300	310	310	0.01
3	440	430	440	440	0.01
5	650	620	600	410	0.22
7	730	700	670	390	0.03

Table 2. In vitro evaluation of BMSC metabolic activity in 6 mg/mL HA-MA-infused fibrin/HA-MA hydrogels.

Relative fluorescence's	HA-MA Concentration				P value
days	0.0	0.5	1	1.5	
1	280	290	300	300	0.02
3	430	420	440	440	0.03
5	640	610	590	400	0.33
7	720	690	660	380	0.01

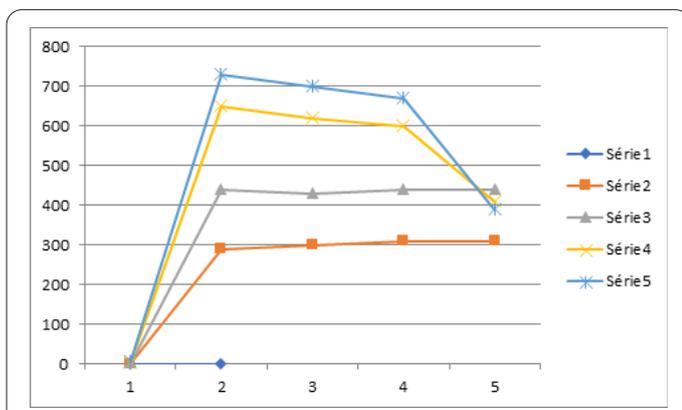


Figure 2. In vitro evaluation of BMSC metabolic activity in 4 mg/mL HA-MA-infused fibrin/HA-MA hydrogels.

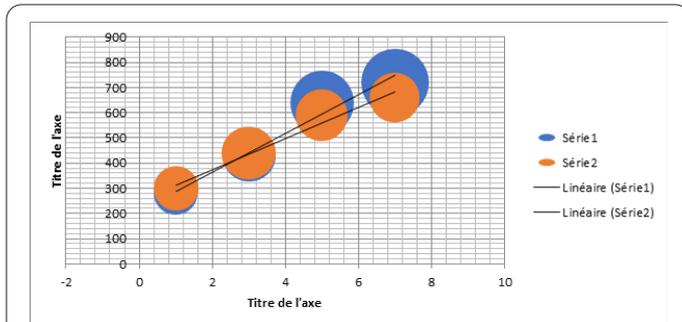


Figure 3. In vitro evaluation of BMSC metabolic activity in 6 mg/mL HA-MA-infused fibrin/HA-MA hydrogels.

BMSC, the gel was able to become more compact.

Live/dead labelling also indicated a rise in the number of viable cells and cell density at successive time intervals for both 4 mg/mL and 6 mg/mL fibrinogen concentrations, indicating that the BMSC was alive and well. Indeed, this held true for both of the aforementioned concentrations. Both at 4 mg/mL and 6 mg/mL, the number of highlighted cells was larger on day 6 compared to day 2. Both situations have this feature. Both the metabolic test and the live/dead staining methods give a useful index of cellular growth. Cell proliferation is strongly suggested by the presence of increased cell density, as measured by live/dead staining, and by quantitative measurements of cellular activity.

But metabolic activity alone is not sufficient to ensure cell division. Recent studies have shown that a decrease in cellular proliferation may be connected with an increase in the mechanical strength of hydrogels with a similar structure. There was a decrease in endothelial cell proliferation and an increase in mechanical strength in fibrin scaffolds that included HA after treatment with tyramine and crosslinking in the presence of hydrogen peroxide.

Mechanical and structural characterization of fibrin/HA-MA hydrogels

In order to analyse the fibrin/HA-MA hydrogels' mechanical rigidity, unconfined compression testing was performed (Table 3, Fig 4 and B, Fig 5), which resulted in the determination of the compressive modulus at 20% strain. The data, when analysed using a one-way analysis of variance (ANOVA), revealed statistically significant differences between the groups (p 0.05).

The Student's t-Test was used to determine the p-value between the two groups in order to determine whether or not there were differences between the groups that were statistically significant (p 0.05). The range of the compressive modulus of fibrin/HA-MA hydrogel increased from 1.90 ± 0.30 kPa to 4.30 ± 0.98 kPa when the HA-MA content was varied from 0 to 1.5 mg/mL. When the fibrinogen concentration was increased to 6 mg/mL, the compressive modulus increased from 3.50 ± 0.50 kPa to 6.70 ± 0.88 kPa. There was no evidence to suggest that one of the two situations was significantly different from the other. We decided to dismiss the insignificant apparent difference when analysing the compressive moduli of fibrinogen at 4 mg/mL with 0 mg/mL of HA-MA against fibrinogen at 4 mg/mL with 0.5 mg/mL of HA-MA (p = 0.25). This was because the difference was not statistically significant. The fibrinogen hydrogel with a compressive modulus of 6 mg/mL was the most robust among all HA-MA concentrations. The quantity of fibrinogen and HA-MA produced an increase in compressive modulus that was directly proportional to that increase.

Evaluation of mRNA Expression

In order to evaluate the chondrogenic capacity of the cells, they were placed in an as-selected hydrogel for a

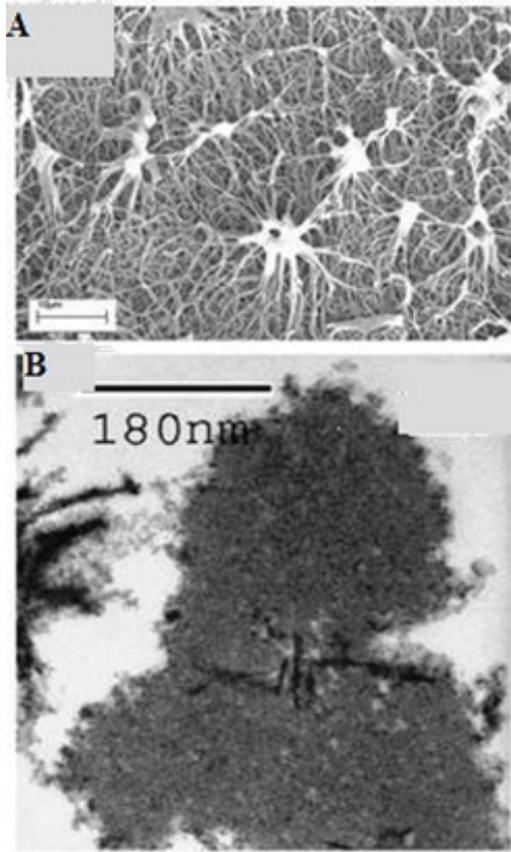


Figure 4. A: structural characterization of fibrin/HA-MA hydrogels; B: At 180nm, the structural characterization.

is difficult to treat due to the complexity of the affected tissue. Conservative treatment often yields the best results for paediatric patients, while surgical options are typically reserved for application to adult patient groups(13-15). Up to fifty percent of all instances of acute ankle sprains and fractures have a chance of causing injury to the osteochondral tissue. This is a potential (OCL). Pain, edema, and stiffness are the three symptoms that manifest themselves in individuals who have OLTs more often than any others. These symptoms often appear while the patient is engaging in strenuous physical activity, such as during a sporting event; however, they almost never appear when the patient is at rest (16). There is also the chance that mechanical symptoms, such as locking and catching, could be present in certain circumstances. This is something that might be the case. Patients often ascribe the pain they are feeling to either a single traumatic incident or a history of reoccurring sprains. This may be quite misleading for the treating physician (17).

Plain radiography, magnetic resonance imaging (MRI), and computed tomography (CT) are the imaging modalities that are used in the clinic for the identification and diagnosis of OLTs at the most common frequency. The many imaging modalities each come with their own individual sets of benefits, drawbacks, and categorization schemes. In most cases, a plain radiograph will serve as the first step in the diagnostic process. Radiographs of the foot and ankle in the AP mortise and lateral weight-bearing positions are routine practice. Radiographs of the foot and ankle

period of 12 days and subjected to an incubation process. Quantitative polymerase chain reaction (qPCR) was used throughout this time period to analyse the levels of gene expression for collagen type 1 alpha 1 (COL1A1), collagen type 2 alpha 1 (COL2A1), SOX9, and aggrecan (ACAN) (Table 4-6) As a result of the fact that platelet lysate (PL) is known to include growth factors such as transforming growth factor (TGF), which are known to induce chondrogenesis, it was used as a supplementation in order to emulate real-life applications that make use of BMSCs. A platelet lysate was used in order to accomplish this task. The findings of a simple analysis of variance revealed that the data in the set used for the comparison were statistically distinct ($p < 0.05$). The standardisation of the fold increase in mRNA transcripts was done on cells that were enclosed inside fibrin/HA-MA gels that contained 10% foetal bovine serum (FBS) (Tables 4-6 and Fig 6).

Discussion

Despite the fact that osteochondral lesions of the talar dome are becoming more widely recognised, the problem

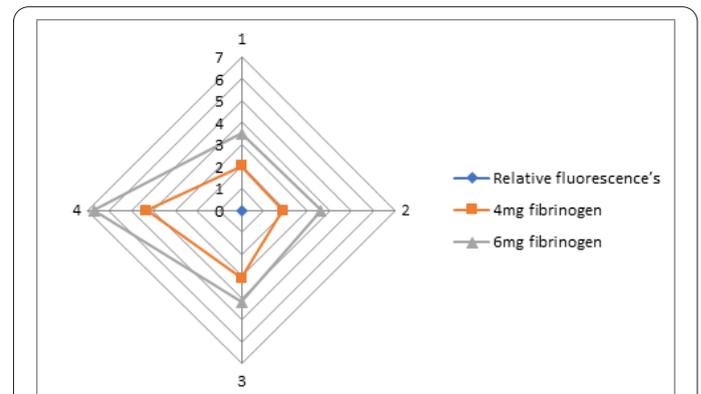


Figure 5. Fibrin/haemogel/methacrylate hydrogels' compressive modulus at 20% strain.

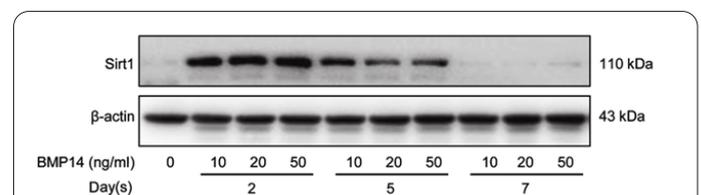


Figure 6. mRNA expression for BMSCs.

Table 3. Fibrin/haemogel/methacrylate hydrogels' compressive modulus at 20% strain.

Relative fluorescence's	HA-MA Concentration			
	0.0	0.5	1	1.5
4mg fibrinogen	2.0±0.41	1.90±0.30	3.10±0.81	4.30±0.98
6mg fibrinogen	3.50±0.50	3.60±0.77	4.20±0.74	6.70±0.88
p-value	0.32	0.25	0.01	0.02

Table 4. Gene sequences for the production of mRNA throughout the process of chondrogenesis.

Chondrogenic marker	Symbols	mRNA Sequence	
		Left primer	Right primer
Aggrecan	ACAN	cctccccttcacgtgtaaaa	gctccgctctgtagtctgc
Glyceraldehyde-3-Phosphate Dehydrogenase [†]	GAPDH	tagtagccgggcctacttt	tcctcctgtttcatccaage
Collagen Type 2 Alpha 1	COL2A1	agggccaggatgtccatt	aggagagggcccacagag
Collagen Type 1 Alpha 1	COL1A1	gggattccctggacctaag	ggaacacctgcctcca
SRY-box containing gene 9	SOX9	gtaccgcacttgcaaac	tctcgtctcgttcagaagtc

Table 5. mRNA expression for BMSCs.(collagen type 1 alpha 1 gene).

Collagen type 1 alpha 1 gene	Mean \pm sd	P value
Fibrin/HA-MA FBS	1.11 \pm 0.07	0.01
Fibrin only PL	0.91 \pm 0.06	0.02
Fibrin/HA-MA	0.55 \pm 0.04	0.01

Table 6. mRNA expression for BMSCs.

SOX9 gene	Mean \pm sd	P value
Fibrin/HA-MA FBS	1.44 \pm 0.36	0.02
Fibrin only PL	2.21 \pm 0.98	0.03
Fibrin/HA-MA	15.85 \pm 1.36	0.04

in other positions are not standard practice. The trauma series is a typical course of treatment that is administered in the event of serious injuries. Due to its greater capacity to view soft tissues such as articular cartilage, synovium, and tendons, magnetic resonance imaging (MRI) gives an edge in terms of locating and characterising OLTs (18).

When plain radiographs are unable to establish a definitive diagnosis or when symptoms persist, this imaging modality is the one that is most often used. According to the 5-stage Hepple MRI categorization scheme for OLTs, stage 1 indicates that there is only an injury to the articular cartilage, stage 2 indicates that there is damage to the cartilage with a subchondral fracture, and stage 3 indicates that there is damage to the articular cartilage with an osteochondral fracture. It is the existence of oedema that decides whether the disease is in stage 2a, which is acute, or stage 2b, which is chronic. Stages 3 and 4 of osteochondritis dissecans are distinguished from one another by the presence of detached osteochondral fragments that are neither displaced nor displaced. Subchondral cysts are present when a patient is at the stage 5 level (19).

In the most recent decade, the biological adjuvant known as BMAC has gained increased popularity for usage in one-step cartilage repair treatments such as OLTs. These techniques include ones that do not need any further steps (20). BMAC contains a wide variety of stem cells and progenitor cells, two examples of which are mesenchymal stem cells (MSCs) and human progenitor cells (HPCs). Both of these kinds of cells have the potential capability to heal damaged hyaline cartilage (21). An extraction of bone marrow is taken from the iliac crest as part of the BMAC procedure. White cells from the bone marrow, including MSCs, HPCs, and the rest of the immune cell fractions, are concentrated with the help of commercial kits before being injected into the patient's OLT in conjunction with a biological scaffold consisting of hyaluronic acid and fibrin gel. This process is called autologous bone marrow transplantation (ABMT) (22). It has been shown beyond a reasonable doubt that the use of BMAC is successful in

the treatment of osteochondral defects of the ankle over an extended period of time.

Hematopoietic cells have the ability to differentiate into platelets, which may contain a variety of growth factors, while mesenchymal stem cells have the capability to differentiate into chondrocytes and undergo the process of chondrocyte differentiation (23). Platelets and mesenchymal stem cells can be found in cBMA, and these cells may contribute to improved remodelling of the subchondral bone, as well as increased levels of type-II collagen and proteoglycan differentiation of fibrocartilage. This can result in the formation of a more hyaline-like tissue that is more durable. The aspirate that is obtained from the ilium may be injected at the defect area under the guidance of an arthroscope as part of the arthroscopic bone marrow stimulation procedure. It is possible that the harvested tissue may be immersed in cBMA throughout the process of restoring injured tissue. This tissue will then be used to both backfill the donor site and replace the damaged tissue (24).

The purpose of an arthroscopy examination might be expressed as In order to enable mesenchymal stem cells that are capable of creating fibrocartilage repair tissue to migrate into the lesion, it is necessary to stimulate the bone marrow. The bone marrow will be able to penetrate the subchondral bone as a result of this. When the osteochondral fragment has been located and probed, the damaged component is removed, and marrow stimulation is accomplished by either drilling into the area or making use of a microfracture awl. Many holes are drilled into the subchondral bone, which leads to the development of a fibrin clot and the discharge of cytokines and growth factors from the vascularized subchondral bone (25).

As a result, it is feasible for pluripotent mesenchymal stem cells to amass at the site of the lesion, differentiate into cells that are similar to chondrocytes, and produce repair tissue that produces type II collagen in response to growth stimuli (26). It is suggested that each microfracture be formed at a distance of three to four millimetres and at a depth of approximately three millimetres (27-30).

When the size of the opening in the subchondral bone is increased, the number of mesenchymal stem cells that are brought in also rises. In addition, bone marrow aspirates concentration delivery systems that utilise a porcine collagen matrix and hyaluronic acid membranes have been developed. These systems have been successful in treating patients. (28-30).

Conclusion

We concluded that the utilisation of bone marrow concentration in conjunction with a combination of fibrin and Hyaluronan treatment is safe for patients suffering from OCD of the ankle and is well tolerated by these patients as both a primary treatment and a non-primary treatment option.

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