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ABSTRACT

The invention provides an autologous stem cell containing osteoinductive formulation useful for healing bone injuries and its method of preparation. The osteoinductive formulation described for healing the bone injuries is a combination of autologous mesenchymal and hematopoietic stem cells, platelet rich plasma containing mononuclear cells with growth factors and scaffolding compounds. Mesenchymal and hematopoietic stem cells used in the osteoinductive formulation are isolated from bone marrow and/or adipose tissue. Platelet rich plasma containing mononuclear cells with growth factors is isolated from peripheral blood. The invention also provides a Minimal/Non-invasive procedure for the treatment of Avascular Necrosis by administrating the osteoinductive formulation.

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For Dr. Pradeep Mahajan

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IN/PA-2013

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CLAIMS

I claim,

1. A method for preparation of therapeutically effective dose of an osteoinductive formulation for treating necrotic section of the bone comprising the steps of,
   A) Isolation of patient's own bone marrow and/or adipose cells containing stem cells;
   B) Isolation of a patient's own platelet rich plasma (PRP) containing mononuclear cells with growth factors;
   C) Combining stem cells containing bone marrow and/or adipose tissue isolated in (a) and platelet rich plasma isolated in (b) with a scaffolding material and bone marrowserumin a therapeutically effective concentration,

   Wherein the no of bone marrow cells and adipose cells in the Osteoinductive formulation is determined based on the grade of the necrosis and Body Mass Index (BMI)

2. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the stem cells present in the bone marrow cells are haematopoietic stem cells and mesenchymal stem cells, Adult multipotent stem cells and very small embryonic like stem cells (VSEL).
3. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the growth factors present in the platelet rich plasma include VEGF, HGF, FGF, PDGF, TGF-β, IGF-1, IGF-2, EGF, CTGF and combination thereof.
4. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the mononuclear cells present in the platelet rich plasma

includelymphocyte population containing T-cells, B-cells, Endothelial Cells, Dendritic cells, NK cells, Macrophages, Monocytes, Basophil, Neutrophil, Eosinophil, Plasma cells, Granulocytes, Hematopoietic progenitor cells and Platelets.

5. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the stem cells containing adipose tissue is isolated from lower and upper abdomen regions or gluteal region of lower and upper buttock.
6. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the isolation of stem cells containing bone marrow cells is done from anterior and posterior iliac crest, tibia.
7. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the platelet rich plasma is derived from peripheral blood obtained from any accessible vein.
8. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the scaffolding material used is selected from the group of thrombin, calcium chloride, Sodium bicarbonate, collagen, fibrin, alginate, hyaluronic acid, chitosan, Polylactic co-glycolic acid, PEG, protein based biomaterials, synthetic biomaterials, polysaccharide based biomaterials and peptide based biomaterials, ceramic based biomaterialsor combination thereof.
9. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the noof bone marrow cells is in the range of 50-100 X 10^6, 150-200X 10^6, 250-300X 10^6, 350-400X 10^6cells for grade 0-I, I, II, III, IV necrosis respectively.
10. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the no of adipose cells is in the range of 1600-2000X 10^6, 1800-2500 X 10^6, 2500-3500 X 10^6, 4000-5000 X 10^6cells for grade 0-I, I, II, III, IV necrosis respectively.

11. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the volume of bone marrow serum is 2-5 ml.
12. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the scaffolding material is Calcium chloride.
13. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the scaffolding material is Hyaluronic acid.
14. A method for preparation of an osteoinductive formulation as recited in claim 1 and 20 wherein, the volume of calcium chloride is 10-15% of Platelet Rich Plasma volume.
15. A method for preparation of an osteoinductive formulation as recited in claim 1 and 13 wherein, the volume of Hyaluronic acidis 1-2 ml.
16. A therapeutically effective dose of an Osteoinductive formulation for treating necrotic sections of the bone comprising,

A) Stem cells containing patient's own bone marrow and/or adipose cells;
B) Platelet rich plasma containing mononuclear cells with growth factors;
C) One or more scaffolding material or combination thereof;
D) Bone marrow serum;

Wherein the no of bone marrow cells and/or adipose cells in the Osteoinductive formulation is determined based on the grade of the necrosis and BMI.

17. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the stem cells present in the bone marrow cells are haematopoietic stem cells and mesenchymal stem cells.
18. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the growth factors present in the platelet rich plasma include VEGF, HGF, FGF, PDGF, TGF-β, IGF-1, IGF-2, EGF, CTGF and combination thereof.

19. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein lymphocyte population containing T-cells, B-cells, Endothelial Cells, Dendritic cells, NK cells, Macrophages, Monocytes, Basophil, Neutrophil, Eosinophil, Plasma cells, Granulocytes, Hematopoietic progenitor cells and Platelets.
20. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the stem cells containing adipose cells are isolated from lower and upper abdomen regions or gluteal region of lower and upper buttock.
21. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the isolation of bone marrow cells is done from bone marrow aspirate obtained from anterior and posterior iliac crest, tibia.
22. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the platelet rich plasma is derived from peripheral blood obtained from any accessible vein.
23. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the scaffolding material used is selected from the group of thrombin, calcium chloride, Sodium bicarbonate, collagen, fibrin, alginate, hyaluronic acid, chitosan, Polylactic coglycolic acid, PEG, protein based biomaterials, synthetic biomaterials, polysaccharide based biomaterials and peptide based biomaterials, ceramic based biomaterials or combination thereof.
24. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the concentration of bone marrow cells containing the stem cells is in the range of 50-100 X 10^6, 150-200 X 10^6, 250-300 X 10^6, 350-400 X 10^6 cells for grade 0-I, I, II, III, IV necrosis respectively.
25. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the concentration of adipose cells is in the range of

1600-2000 X 10^6, 1800-2500 X 10^6, 2500-3500 X 10^6, 4000-5000 X 10^6 cells for grade 0-I, I, II, III, IV necrosis respectively.

26. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the volume of bone marrow serum is 2-5 ml.
27. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the scaffolding material is Calcium chloride.
28. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the scaffolding material is Hyaluronic acid.
29. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 and 27 wherein, the volume of calcium chloride is 10-15% of Platelet Rich Plasma volume.
30. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 and 28wherein, the volume of Hyaluronic acid is 2-4 ml.
31. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the volume of the dose to be injected in the necrotic site is in the range of 5-20 ml.
32. A method of treating necrotic section of the bone, comprising the steps of:
    A) Isolation of stem cells from patient's own bone marrow or/and adipose cells;
    B) Isolation of a patient's own platelet rich plasma containing mononuclear cells with growth factors;
    C) Combining stem cells isolated in (A) and platelet rich plasma isolated in (B) with a scaffolding material, and Bone marrow serum in a therapeutically effective concentration based on the grade of the necrosis and BMI;
    D) Inserting the said therapeutically effective concentration of an osteoinductive formulation into the said necrotic section.

33. A method of treating necrotic section of the bone as recited in claim 32wherein, the stem cells present in the bone marrow are haematopoietic stem cells and mesenchymal stem cells, Adult multipotent stem cells and VSEL.
34. A method of treating necrotic section of the bone as recited in claim 32 wherein, the growth factors present in the platelet rich plasma include VEGF, HGF, FGF, PDGF, TGF-β, IGF-1, IGF-2, EGF, CTGF and combination thereof.
35. A method of treating necrotic section of the bone as recited in claim 32 wherein, lymphocyte population containing T-cells, B-cells, Endothelial Cells, Dendritic cells, NK cells, Macrophages, Monocytes, Basophil, Neutrophil, Eosinophil, Plasma cells, Granulocytes, Hematopoietic progenitor cells and Platelets.
36. A method of treating necrotic section of the bone as recited in claim 32 wherein, the stem cells containing adipose cells are isolated from lower and upper abdomen regions or gluteal region of lower and upper buttock.
37. A method of treating necrotic section of the bone as recited in claim 32 wherein, the isolation of bone marrow cells is done from bone marrow aspirate obtained from anterior and posterior iliac crest, tibia.
38. A method of treating necrotic section of the bone as recited in claim 32 wherein, the platelet rich plasma is derived from peripheral blood obtained from any accessible vein.
39. A method of treating necrotic section of the bone as recited in claim 32 wherein, the scaffolding material used is selected from the group of thrombin, calcium chloride, Sodium bicarbonate, collagen, fibrin, alginate, hyaluronic acid, chitosan, Polylactic coglycolic acid, PEG, protein based biomaterials, synthetic biomaterials, polysaccharide based biomaterials and peptide based biomaterials, ceramic based biomaterials or combination thereof.

40. A method of treating necrotic section of the bone as recited in claim 32 wherein, the concentration of bone marrow cells containing the stem cells is in the range of 50-100 X 10^6, 150-200 X 10^6, 250-300 X 10^6, 350-400 X 10^6 cells for grade 0-I, I, II, III, IV necrosis respectively.
41. A method of treating necrotic section of the bone as recited in claim 32 wherein, the concentration of adipose cells is in the range of 1600-2000 X 10^6, 1800-2500 X 10^6, 2500-3500 X 10^6, 4000-5000 X 10^6 cells for grade 0-I, I, II, III, IV necrosis respectively.
42. A method of treating necrotic section of the bone as recited in claim 32 wherein, the volume of bone marrow serum is 2-5 ml.
43. A method of treating necrotic section of the bone as recited in claim 32 wherein, the scaffolding material is Calcium chloride.
44. A method of treating necrotic section of the bone as recited in claim 32 wherein, the scaffolding material is Hyaluronic acid.
45. A method of treating necrotic section of the bone as recited in claim 32 wherein and 43 wherein, the volume of calcium chloride is 10-15% of Platelet Rich Plasma volume.
46. A method of treating necrotic section of the bone as recited in claim 32 and 44 wherein, the volume of Hyaluronic acid is 1-2 ml.
47. A method of treating necrotic section of the bone as recited in claim 32 wherein, the volume of the dose to be injected in the necrotic site is in the range of 5-20 ml.

Dated 15th Day of April 2015

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FIELD OF THE INVENTION

The present invention deals with the field of tissue engineering. More specifically the present invention relates to an autologous stem cell containing Osteoinductive formulation useful for healing bone injuries and its method of preparation. The Osteoinductive formulation described for healing the bone injuries is a combination of autologous mesenchymal and hematopoietic stem cells, platelet rich plasma containing mononuclear cells with growth factors and scaffolding compounds. Mesenchymal and hematopoietic stem cells used in the Osteoinductive formulation are isolated from bone marrow and/or adipose tissue.The invention relates to delivery of Osteoinductive formulation with minimally invasive technology. Platelet rich plasma containing mononuclear cells with growth factors is isolated from peripheral blood.

BACKGROUND OF THE INVENTION

Avascular necrosis (AVN) also referred to as Osteo necrosis, aseptic necrosis of bone, bone infarct or ischemic necrosis of bone, is the death of bone caused by an impaired blood supply. AVN is not a specific disease but a condition in which there is death of a localized area of bone. More than 2 million people suffer from Avascular Necrosis (AVN) around the world each year, with about 40,000 cases been reported in India itself. In the United States, approximately 15,000 new cases of AVN are reported each year. AVN accounts for more than 10% of total hip replacement surgeries performed in the United States. AVN has become a disease of young age that generally affects people between the ages of 20-40 and is bilateral in 55% of cases.

There are generally two categories of AVN: traumatic and nontraumatic. The most frequent cause of traumatic AVN is a displaced fracture, most often affecting hip. It damages the blood vessels supplying the upper thigh of the bone resulting in death of

the bone. This type of trauma-related avascular necrosis affects about 20 percent of people who dislocate a hip joint.

The Ficat classification is a commonly used system to stage AVN of the hip, and uses a combination on plain film, MRI and clinical features:

Stage 0:

- plain film: normal
- MRI: normal
- clinical symptoms: nil

Stage I:

- plain film: normal or minor osteopenia
- MRI: oedema
- bone scan: increased uptake
- clinical symptoms: pain typically in the groin

Stage II:

- plain film: mixed osteopenia &/or sclerosis &/or subchondral cysts, without any subchondral lucency (crescent sign: see below)
- MRI: geographic defect
- bone scan: increased uptake
- clinical symptoms: pain and stiffness

Stage III:

- plain film: crescent sign & eventual cortical collapse
- MRI: same as plain film
- clinical symptoms: pain and stiffness+/- radiation to knee and limp

Stage IV:

- plain film: end stage with evidence of secondary degenerative change
- MRI: same as plain film
- clinical symptoms: pain and limp

In view of the advances in technology, various approaches to treat avascular necrosis have been developed. The important and relevant methods are discussed below;
Several nonsurgical measures are available for treating the symptoms caused by osteonecrosis. Taking anti-inflammatory drugs or other pain relievers, minimizing activity and stress (such as weight bearing for osteonecrosis of the hip and knee), and undergoing physical therapy are ways to relieve symptoms but not cure the disorder or change its course. Non-steroidal anti-inflammatory drugs (NSAIDS) such as naproxen and osteoporosis drugs such as alendronate provide temporary relief to the patient. Long term use of NSAIDs leads to kidney and liver damage, as well as gastric bleeding. Though such drugs help relieve the pain, they act only temporarily and are unable to halt the progression of the disease. Various anti-cholesterol drugs as well as blood thinners such as warfarin are suggested in case of increased fat levels in blood and presence of blood clots. Modalities such as pulsed electromagnetic field and acoustic waves have been used to relieve symptoms caused by AVN.

There are a number of surgical procedures that slow progression of the disorder. These are most effective for treating early disease, particularly of the hip, that has not yet progressed to bone collapse.

Core decompression is one of the simplest and common procedures performed for treatment of AVN, where the internal bone pressure is relieved by drilling a hole into the bone, an 8-10mm cylindrical core of bone is removed from the antero-lateral segment of the femoral head, which creates an open cylindrical channel Intra operative and post-operative pain is large and unbearable. Such microsurgical

procedures require specialized training, equipment and expertise. Long hospitalization of more than a week is involved, risk of infections and chances of blood clot formation after surgery are also present. Patients require the use of crutches for almost two months.

To treat AVN, bone grafts along with core decompression have also been performed. The open channel may be filled with either a vascularized or a non-vascularized bone graft. but this often causes weakness in the bones in the area they are implanted and in the neck region, and the treatment is a very complex and time consuming operating procedure with high cost factor. The area from where the bone grafts are obtained becomes weak and can fracture and even gets infected. In case of allogenic grafts, there is high chance of rejection. Bone grafts do not provide sufficient mechanical strength to the joint. Bone grafts are unsuccessful in treatment of advanced stages of AVN.

In the early stages of AVN, bone reshaping also known as osteotomy is a common surgical Technology where the bones are reshaped or partially removed to realign the load-bearing surfaces of the joint. Various types of proximal femoral osteotomies have been used in the treatment of avascular necrosis of the femoral head over the past thirty years. But in such procedures fractures of the femur bone can take place. Recovery is complicated and takes time, involving painful physiotherapy. Joint movement is restricted and painful, with patient having to use crutches for 4-6 weeks, and only able to walk normally after 2-3 months. Patient cannot follow his old lifestyle freely and has to take certain precautions throughout his lifetime. But such a procedure does not guarantee a complete recovery from AVN, and the disease eventually progresses to a more advanced stage. Procedures such as bone grafting and osteotomies are technically demanding, require protected weight bearing for up to 6 months, and have not been done often in the US. Reports vary as to their

indications and effectiveness. They should be done primarily at selected centres that have the surgical experience and facilities to achieve optimal results.

When AVN has advanced to grade III and IV, the patient has no option but to undergo THR. Total Hip Replacement (THR) is a common option for treatment of AVN where the joint is replaced by a prosthetic implant which can be metallic or synthetic. AVN accounts for approximately 10% of the total hip replacements performed. Such a procedure is generally a long supramajor surgery costing about 4-5 lac rupees, that requires major anaesthesia, hospitalization of more than a week and major surgery risk. Patient has to be put on warfarin to avoid post-surgical complications such as deep vein thrombosis and pulmonary embolism which can be life-taking. Implants are also short-lived about 10-15 years and in young people, another hip surgery will be required which again carries its own set of complications and displacement of implant requires revision surgery which involves more complications, metallic implants are said to release ions into the surrounding tissue, leading to its inflammation. Due to presence of metallic implants, patient cannot undergo MRI scans due to magnetic field. Recovery from THR is complicated, patient undergoes painful physiotherapy. This also causes metal toxicity and results into chronic arthritis. Bending of hip more than 90° is impossible. Deterioration around implant and joint occurs leading to inflammation and infection. Regular movements have to be done cautiously due to fear of dislocating the implant. Simple activities such as driving a car, playing sports are restricted. While sleeping patient cannot lie on one side, crossing over legs also is limited, as well as sitting on low chair and toilet seats. Sexual activities are also barred due to risk of causing damage to the operated join or displacing it.

Alternatives to total hip replacement include surface replacement arthroplasty (SRA) and hemi-SRA. SRA involves the insertion of two metal caps, one into the acetabulum and one onto the femoral head, producing a metal-on-metal articulation.

Hemi-SRA involves placement of a metal cap onto only the femoral head. It is done only if disease is limited to the femoral head and is considered a temporizing procedure. These procedures are done less often now than a few years ago because of an increasing incidence of local complications, prosthesis failure, and concerns about possible long-term systemic effects of metal ions.

Even though the above surgical procedures slow the progression of AVN, they cannot stop the condition completely neither cure it. Post-surgical problems are numerous. Patients do not regain the natural flexibility of joints they had before developing AVN. Playing sports and activities such as running, riding is risky. They have to be cautious about all their everyday movements. Sexual activities are also highly restricted due to risk of causing damage to the operated joint, leading to discontent in the patient's life. Patients cannot go back to their old lifestyle again, due to the fear of causing damage to their operated joint or dislocating it.

Patent application number 3727/MUM/2012 deals with treatment of avascular necrosis with mesenchymal stem cells and platelet rich plasma, with core decompression involved. Since core decompression is a surgical procedure, anaesthesia use is involved resulting into hospitalization for greater than 48hrs.Risk of fracture is associated with core decompression and weakening of the bone and head, osteopenic bone.

Most of the therapies discussed above deal with surgical procedures that have their own complications, use ofanaesthesiaand hospitalization of more than 7 days. The complex procedures have high cost. Often such surgeries fail, requiring second surgeries in case of misplaced or worn off implants. Post-surgical complications are also numerous including deep vein thrombosis, infections. Regular check-upsare needed to be done to check the state of the implant Bending of hip more than 90° is impossible. Patient has to use crutches for about 4-6 weeks and can walk normally only after 2-3 months. Life of the patient remains complicated due to lack of

complete mobility. Patient has to remain careful of all the activities and movements he carries out. He cannot regain his old lifestyle.
Therefore a need exists for a system and method for treating avascular necrosis in a manner that is simpler and involves fewer complications.

OBJECTIVE OF THE INVENTION

The primary objective of this invention is to provide a minimum invasive non-surgical technology for the treatment of Avascular Necrosis (AVN).

Another objective of the present invention is to provide a non-surgical technology for the treatment of Avascular Necrosis which does not involve use of general anaesthesia.

Another objective of the present invention is to provide an autologous stem cell containing Osteoinductive formulation useful for healing bone injuries including Avascular Necrosis (AVN).

Another objective of the present invention is to provide a process of preparation of an autologous stem cell containing Osteoinductive formulation useful for healing bone injuries including Avascular Necrosis (AVN).

Another objective of the present invention is to provide a process of preparation of an autologous stem cell containing Osteoinductive formulation as per the stage of the avascular necrosis to be treated.

Another objective of the present invention is to provide a minimally invasive Technologyfor the treatment of Avascular Necrosis with maximum hospitalization period of only 1-3 days.

Another objective of the present invention is to provide a minimally invasive Technology to neovascularizationof the necrotic area of bone/cartilage.

Another objective of the present invention is to provide a minimally invasive Technology to regain normal intraosseous pressure due to neovascularization of bone.

Another objective of the present invention is to provide a minimally invasive Technology for providing complete relief from Avascular Necrosis to the patient.

Another objective of the present invention is to provide a minimally invasive Technology for restoring the joint to its original form, its flexibility and mobility, along with strength and efficiency.

Another objective of the present invention is to provide a minimally invasive Technology for treatment of avascular necrosis that is entirely cost-effective and economical without any post-surgicalcomplications.

SUMMARY OF THE INVENTION

The invention provides an autologous stem cell containing Osteoinductive formulation useful for healing bone injuries and its method of preparation. The Osteoinductive formulation described for healing the bone injuries is a combination of autologous mesenchymal and hematopoietic stem cells, platelet rich plasma containing mononuclear cells with growth factors and scaffolding compounds. Mesenchymal and hematopoietic stem cells used in the Osteoinductive formulation are isolated from bone marrow and/or adipose tissue. Platelet rich plasma containing mononuclear cells with growth factors is isolated from peripheral blood. The invention also provides a Minimal/Non-invasive technology for the treatment of Avascular Necrosis by delivering the osteoinductive formulation.

Another embodiment of the present provides a Technologyfor using mesenchymal and hematopoietic stem cells developed for treatment of AVN. These stem cells are autologous in nature, as they are isolated from the patient's own body. This

Technology is minimally invasive, with no risk factors or complications, hospital stay of only 2-3 days and no post-surgical complications. Due to the ability of stem cells to replace necrotic tissue by neoangiogenesis, the damaged bone is naturally repaired in a period of over 1-3 months depending on the grade of the disease. This Technology provides a permanent treatment modality for AVN.

Another embodiment of the present invention provides a Technology for preparation of theosteoinductive formulation for treatment of avascular necrosis wherein the amount of active components like Bone marrow cells (stem cells), Platelet rich plasma and Adipose cells of the said osteoinductive formulation is based on the grade of the avascular necrosis.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a cross-sectional view of the hip joint of a person having a femoral head with a necrotic section.

Figure 2 is the cross sectional view of Figure 1 after having undergone the treatment method of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

One embodiment of the present invention provides a Minimal/Non-invasive technology for the treatment of Avascular Necrosis (AVN), in the present invention major emphasis was given on minimally invasive technology for the treatment and complete recovery from AVN of bone/joint.

The method of the present invention for the treatment of AVN involves use of Haematopoietic and Mesenchymal stem cells, other autologous AMSC cell types and growth factors along with biological scaffolding materials.

Main features of the method disclosed in the present invention are.

a) Minimally invasive, non-operative and outdoor technology for the treatment of AVN.
b) Complete regeneration of necrotic region of the bone.
c) Effective utilization of Autologous Mesenchymal and Haematopoieticstem cells along with other autologousgrowth factors in treatment of AVN.
d) Patient should be able to regain strength and functional ability after completion of treatment.
e) The procedure is cost effective and requires minimum hospitalization.
f) Process involves use of autologous cell therapy and has no adverse effects on the patient.
g) No further weakening to the bone/joint and restoration of joint/bone to original structure as well as maintain the original joint with normal strength.

In the present invention efforts have been made to develop minimum and/or Non-Invasive treatment of AVN by using AutologousMesenchymal and Hematopoieticstem cells, Very small embryonic like stem cells, Adult Multipotent stem cells along with the combination of bone marrow concentrate and adipose derived stem cells, platelet rich plasma, chemokines, cytokines and other growth factors along with scaffolding materials. The mesenchymal and Hematopoieticstem cells used in present invention are isolated from bone marrow of patient's body. The said combination helps in repairing the damaged part of bone and thus leads to complete recovery from AVN.The repair is carried out by regeneration of cartilage cells as well as neo-angiogenesis.

Bone Marrow Aspiration and Isolation Process

Stem cells used in the present invention are autologous stem cells that are derived from the patient's bone marrow. To isolate sufficient amount of bone marrow cells, growth factors in the form of subcutaneous injection such as Neulasta and/or Neupogen and/or Neukine and/or Filgrastim are given to patient for 3 subsequent days prior to harvest procedure.

Bone marrow isolation site was swabbed with providone-iodine swab stick followed by spirit. 5 ml of Xylocaine is injected subcutaneously into periosteum. Bone marrow is aspirated from iliac crest using bone marrow aspiration needle.

120 ml of bone marrow aspirate (BMA) was transferred into a centrifugation tube containing Heparin. BMA filter bag is subjected to centrifugation for 14 minutes to concentrate bone marrow cells.Cell concentration is adjusted to get 200-500 millions/ml using physiological saline.

Other methods of isolation of bone marrow cells are use of commercially available kits that are available in market with the following brand names Harvest® and/or Retrieve® and/or Arthrex® and/or ChronOS® and/or Marrowmax®.After the harvest procedure, the area is cleaned thoroughly and bandaged

Adipose cell Isolation

The adipose tissue can be collected by a mini- liposuction procedure from the lower and upper abdomen regions as well as the gluteal region of the thighs and buttocks.

Adipose isolation site was swabbed with providone-iodine swab stick followed by spirit. Adipose is aspirated from gluteal region using liposuction cannula. Adipose aspirate is collected to a sterile 50 ml tube containing Heparin and is incubated at 37^0Celsius for 1 hr followed by centrifugal separation. The separated cells were washed using saline and subjected to cell density analysis. The final cell concentration of adipose was adjusted to 1000-3000 million/cc.

Stromal vascular fraction isolated from adipose tissue which is used for the treatment of AVN and consists of blood endothelial cells, adipocyte progenitors, immune cells, fibroblasts, pericytes and stromal cells.

Platelet Rich Plasma Processing Protocol

Blood isolation site was swabbed with spirit. 100 ml of blood is isolated from peripheral vein and mixed with heparin. The blood is then transferred to a centrifuge tube and is subjected to centrifugation for 14 minutes 1500 RPM to isolate Platelets rich plasma.

In yet another embodiment, platelet rich plasma isolated from peripheral blood is used for treatment of AVN and contains various chemokines, cytokines and growth factors such as VEGF, HGF, FGF, PDGF, TGF-β, IGF-1, IGF-2, EGF and CTGF.

Preparation of Osteoinductive formulation by mixing Adipose cells, Bone marrow derived cells and Platelet rich plasma

The amount of each component in the therapeutically effective dose of Osteoinductive formulation i.e. Adipose cells, Bone marrow derived cells and platelet rich plasma is determined by the stage of the avascular necrosis. The concentration of the each component is as per following table.

Grade of Avascular Necrosis	Bone Marrow cells	Adipose cells	Platelet rich plasma
0-I	50-100 X 10^6	1600-2000 X 10^6	1.5-2.5 X 10^6
II	150-200 X 10^6	1800-2500 X 10^6	
III	250-300 X 10^6	2500-3500 X 10^6	
IV	350-400 X 10^6	4000-5000 X 10^6	

Further component of the formulation are Bone marrow serum 2-5 ml and 1-2 ml Hyaluronic acid (10 mg/ml).Calcium Chloride is used as a scaffolding material/biomaterial ata volume of 10-15% of PRP volume used in the formulation. Other scaffolding materials which can be used are Thrombin, Calcium chloride, Sodium Bicarbonate, Collagen, Fibrin, Alginate, Hyaluronic Acid, Chitosan,

Polylactide co-glycolic acid, other Protein based biomaterials, Synthetic biomaterials, Polysaccharide based biomaterials, peptide based biomaterials, and ceramic based biomaterials or other biomaterials. The final volume of the osteoinductive formulation to be injected in the necrotic section is in the range of 5-20 ml.

Other biomaterials that can also be used with the above mentioned combination are collagen, fibrin, fibrin glue, alginate, hyaluronic acid, chitosan, poly lactic-co-glycolic acid, polyethylene glycol, protein based biomaterials, synthetic biomaterials, Polysaccharide based biomaterials, Peptide based biomaterials, and Ceramic based biomaterials or any other biomaterials available or combination thereof.

Other factors considered while formulating the dosage of the Osteoinductive formulation are the age and body mass index (BMI) of the patient as well as the grade of damage/disease.

Delivery of therapeutically effective dose in affected area

The process of delivery therapeutically effective dose of Osteoinductive formulation is performed under C-Arm (High Frequency) using spinal needle of 18 Bore. Under local anaesthesia, the puncture using 18-gauge needle is done to reach to the site of necrosis of the head of the femur. Local anaesthesia is given after identification of the puncture point under c-arm control. This is also done after identifying the location of the femoral artery. Preferably a point is selected away from the femoral artery to reach to the site of necrosis under C-Arm control. Puncturing location is identified.

Alternative locations where such an intra-articular injection is most commonly given are the posterior and anterior approach to the femoral head and the medial/lateral tenth above mentioned treatment can be given more than once in a month and can be done every month/alternate monthup to six months.

An intravenous dose of the same is also given to the patient through normal saline drip, followed by an observation period of 0-48 hours.

After the dose is given, the patient is generally given certain antibiotics intravenously such as Piptaz and/or and/or Galpara and/or Monocef and/or Tramedol and/or Ceptazidine.

Post transplantation procedure treatment and precautions

Post transplantation procedure, various medicines are advised such as Nutraceuticals calcium in the form of osteophos and Catrizen Forte, multivitamins, amino acid supplements.

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CLAIMS

I claim,

1. A method for preparation of therapeutically effective dose of an osteoinductive formulation for treating necrotic section of the bone comprising the steps of,
   A) Isolation of patient's own bone marrow and/or adipose cells containing stem cells;
   B) Isolation of a patient's own platelet rich plasma (PRP) containing mononuclear cells with growth factors;
   C) Combining stem cells containing bone marrow and/or adipose tissue isolated in (a) and platelet rich plasma isolated in (b) with a scaffolding material and bone marrowserumin a therapeutically effective concentration,

   Wherein the no of bone marrow cells and adipose cells in the Osteoinductive formulation is determined based on the grade of the necrosis and Body Mass Index (BMI)

2. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the stem cells present in the bone marrow cells are haematopoietic stem cells and mesenchymal stem cells, Adult multipotent stem cells and very small embryonic like stem cells (VSEL).
3. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the growth factors present in the platelet rich plasma include VEGF, HGF, FGF, PDGF, TGF-β, IGF-1, IGF-2, EGF, CTGF and combination thereof.
4. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the mononuclear cells present in the platelet rich plasma

includelymphocyte population containing T-cells, B-cells, Endothelial Cells, Dendritic cells, NK cells, Macrophages, Monocytes, Basophil, Neutrophil, Eosinophil, Plasma cells, Granulocytes, Hematopoietic progenitor cells and Platelets.

5. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the stem cells containing adipose tissue is isolated from lower and upper abdomen regions or gluteal region of lower and upper buttock.
6. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the isolation of stem cells containing bone marrow cells is done from anterior and posterior iliac crest, tibia.
7. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the platelet rich plasma is derived from peripheral blood obtained from any accessible vein.
8. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the scaffolding material used is selected from the group of thrombin, calcium chloride, Sodium bicarbonate, collagen, fibrin, alginate, hyaluronic acid, chitosan, Polylactic co-glycolic acid, PEG, protein based biomaterials, synthetic biomaterials, polysaccharide based biomaterials and peptide based biomaterials, ceramic based biomaterialsor combination thereof.
9. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the noof bone marrow cells is in the range of 50-100 X 10^6, 150-200X 10^6, 250-300X 10^6, 350-400X 10^6cells for grade 0-I, I, II, III, IV necrosis respectively.
10. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the no of adipose cells is in the range of 1600-2000X 10^6, 1800-2500 X 10^6, 2500-3500 X 10^6, 4000-5000 X 10^6cells for grade 0-I, I, II, III, IV necrosis respectively.

11. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the volume of bone marrow serum is 2-5 ml.
12. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the scaffolding material is Calcium chloride.
13. A method for preparation of an osteoinductive formulation as recited in claim 1 wherein, the scaffolding material is Hyaluronic acid.
14. A method for preparation of an osteoinductive formulation as recited in claim 1 and 20 wherein, the volume of calcium chloride is 10-15% of Platelet Rich Plasma volume.
15. A method for preparation of an osteoinductive formulation as recited in claim 1 and 13 wherein, the volume of Hyaluronic acidis 1-2 ml.
16. A therapeutically effective dose of an Osteoinductive formulation for treating necrotic sections of the bone comprising,

A) Stem cells containing patient's own bone marrow and/or adipose cells;
B) Platelet rich plasma containing mononuclear cells with growth factors;
C) One or more scaffolding material or combination thereof;
D) Bone marrow serum;

Wherein the no of bone marrow cells and/or adipose cells in the Osteoinductive formulation is determined based on the grade of the necrosis and BMI.

17. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the stem cells present in the bone marrow cells are haematopoietic stem cells and mesenchymal stem cells.
18. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the growth factors present in the platelet rich plasma include VEGF, HGF, FGF, PDGF, TGF-β, IGF-1, IGF-2, EGF, CTGF and combination thereof.

19. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein lymphocyte population containing T-cells, B-cells, Endothelial Cells, Dendritic cells, NK cells, Macrophages, Monocytes, Basophil, Neutrophil, Eosinophil, Plasma cells, Granulocytes, Hematopoietic progenitor cells and Platelets.
20. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the stem cells containing adipose cells are isolated from lower and upper abdomen regions or gluteal region of lower and upper buttock.
21. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the isolation of bone marrow cells is done from bone marrow aspirate obtained from anterior and posterior iliac crest, tibia.
22. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the platelet rich plasma is derived from peripheral blood obtained from any accessible vein.
23. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the scaffolding material used is selected from the group of thrombin, calcium chloride, Sodium bicarbonate, collagen, fibrin, alginate, hyaluronic acid, chitosan, Polylactic coglycolic acid, PEG, protein based biomaterials, synthetic biomaterials, polysaccharide based biomaterials and peptide based biomaterials, ceramic based biomaterials or combination thereof.
24. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the concentration of bone marrow cells containing the stem cells is in the range of 50-100 X 10^6, 150-200 X 10^6, 250-300 X 10^6, 350-400 X 10^6 cells for grade 0-I, I, II, III, IV necrosis respectively.
25. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the concentration of adipose cells is in the range of

1600-2000 X 10^6, 1800-2500 X 10^6, 2500-3500 X 10^6, 4000-5000 X 10^6 cells for grade 0-I, I, II, III, IV necrosis respectively.

26. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the volume of bone marrow serum is 2-5 ml.
27. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the scaffolding material is Calcium chloride.
28. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the scaffolding material is Hyaluronic acid.
29. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 and 27 wherein, the volume of calcium chloride is 10-15% of Platelet Rich Plasma volume.
30. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 and 28wherein, the volume of Hyaluronic acid is 2-4 ml.
31. A therapeutically effective dose of an Osteoinductive formulation as recited in claim 16 wherein, the volume of the dose to be injected in the necrotic site is in the range of 5-20 ml.
32. A method of treating necrotic section of the bone, comprising the steps of:
    A) Isolation of stem cells from patient's own bone marrow or/and adipose cells;
    B) Isolation of a patient's own platelet rich plasma containing mononuclear cells with growth factors;
    C) Combining stem cells isolated in (A) and platelet rich plasma isolated in (B) with a scaffolding material, and Bone marrow serum in a therapeutically effective concentration based on the grade of the necrosis and BMI;
    D) Inserting the said therapeutically effective concentration of an osteoinductive formulation into the said necrotic section.

33. A method of treating necrotic section of the bone as recited in claim 32wherein, the stem cells present in the bone marrow are haematopoietic stem cells and mesenchymal stem cells, Adult multipotent stem cells and VSEL.
34. A method of treating necrotic section of the bone as recited in claim 32 wherein, the growth factors present in the platelet rich plasma include VEGF, HGF, FGF, PDGF, TGF-β, IGF-1, IGF-2, EGF, CTGF and combination thereof.
35. A method of treating necrotic section of the bone as recited in claim 32 wherein, lymphocyte population containing T-cells, B-cells, Endothelial Cells, Dendritic cells, NK cells, Macrophages, Monocytes, Basophil, Neutrophil, Eosinophil, Plasma cells, Granulocytes, Hematopoietic progenitor cells and Platelets.
36. A method of treating necrotic section of the bone as recited in claim 32 wherein, the stem cells containing adipose cells are isolated from lower and upper abdomen regions or gluteal region of lower and upper buttock.
37. A method of treating necrotic section of the bone as recited in claim 32 wherein, the isolation of bone marrow cells is done from bone marrow aspirate obtained from anterior and posterior iliac crest, tibia.
38. A method of treating necrotic section of the bone as recited in claim 32 wherein, the platelet rich plasma is derived from peripheral blood obtained from any accessible vein.
39. A method of treating necrotic section of the bone as recited in claim 32 wherein, the scaffolding material used is selected from the group of thrombin, calcium chloride, Sodium bicarbonate, collagen, fibrin, alginate, hyaluronic acid, chitosan, Polylactic coglycolic acid, PEG, protein based biomaterials, synthetic biomaterials, polysaccharide based biomaterials and peptide based biomaterials, ceramic based biomaterials or combination thereof.

40. A method of treating necrotic section of the bone as recited in claim 32 wherein, the concentration of bone marrow cells containing the stem cells is in the range of 50-100 X 10^6, 150-200 X 10^6, 250-300 X 10^6, 350-400 X 10^6 cells for grade 0-I, I, II, III, IV necrosis respectively.
41. A method of treating necrotic section of the bone as recited in claim 32 wherein, the concentration of adipose cells is in the range of 1600-2000 X 10^6, 1800-2500 X 10^6, 2500-3500 X 10^6, 4000-5000 X 10^6 cells for grade 0-I, I, II, III, IV necrosis respectively.
42. A method of treating necrotic section of the bone as recited in claim 32 wherein, the volume of bone marrow serum is 2-5 ml.
43. A method of treating necrotic section of the bone as recited in claim 32 wherein, the scaffolding material is Calcium chloride.
44. A method of treating necrotic section of the bone as recited in claim 32 wherein, the scaffolding material is Hyaluronic acid.
45. A method of treating necrotic section of the bone as recited in claim 32 wherein and 43 wherein, the volume of calcium chloride is 10-15% of Platelet Rich Plasma volume.
46. A method of treating necrotic section of the bone as recited in claim 32 and 44 wherein, the volume of Hyaluronic acid is 1-2 ml.
47. A method of treating necrotic section of the bone as recited in claim 32 wherein, the volume of the dose to be injected in the necrotic site is in the range of 5-20 ml.

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For Dr. Pradeep Mahajan

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