Stem Cells

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Introduction

Stem cell engineering is emerging as a promising therapy option in regenerative medicine because of its capability to differentiate into cells of the target tissue (regeneration) and modulate via growth factors to initiate a cascade of healing responses (repair) in the host tissue. When deposited into neuronal, osseous, cartilaginous, and tendinous tissues or muscular defects, stem cells have shown excellent restorative properties through angiogenic, anti-inflammatory, immunomodulatory, antiapoptotic, and antifibrotic mechanisms. While embryonic and induced pluripotent stem cells have unlimited potential to differentiate into various tissues types, the ethical, political, and tumorigenic concerns have limited embryonic and induced pluripotent stem cell engineering, respectively. Furthermore, embryonic stems are fragile and difficult to manipulate. These limitations paved the way for the development of adult mesenchymal stem cell (MSC) technology where MSCs can be harvested from individual patients in abundance and injected into the patient without ethical conflicts and with reduced immunogenic and reduced oncological concerns.

Background and Historical Perspective

The present-day routine bone marrow transplant to treat leukemia began in the 1960s when Mathé demonstrated improved long-term survival in patients with leukemia treated with hematopoietic stem cell treatment. Friedenstein et al. was the first group to observe small deposits of bone- and cartilage-like tissues cultured from MSCs isolated from guinea pig bone marrow and spleen cells in the 1970s. Regardless of where the MSCs are obtained, stem cell regenerations are governed by four fundamental components: viable stem cells, growth factors, scaffolds, and mechanical stimulation of the local environment.

Adult-Derived Mesenchymal Stem Cells

MSCs are adult stem cells that are capable of differentiating into multiple lineages to cause clonal expansion. MSCs are commonly isolated from bone marrow or adipose cells. One of the most well-studied MSCs, also known as stromal stem cells, was first derived from bone marrow. Since the time when MSCs were identified in the bone marrow aspirate, MSC have been harvested ubiquitously throughout the body.

Due to the heterogeneity the cells and their surface markers found in the specimen, International Society for Cellular Therapy defines MSCs as a heterogeneous population of progenitor cells expressing a pattern of characteristic surface markers including:

- CD73, CD90, and CD105 in greater than 95%
- Lacking the expression of hematopoietic markers CD34, CD45, CD14 or CD11, CD79a or CD19, and HLA class II in greater than 95% of the culture

For practical purposes, the three main sources of stem cells are:

- Autologous bone marrow aspirate concentrate (BMAC)
- Autologous stromal vascular fraction (SVF) of adipose tissues
- Allogenic stem cells (e.g., Mesoblast Ltd.) from sources such as bone marrow or adipose cells

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Each stem cell source possesses unique properties (see Table 89.1). Collectively, the number of endogenous MSCs obtained may depend on the individual's age, medical comorbidities, and tissue donor site. MSCs obtained from different tissue types display varied ability to differentiate into specific target tissues. Therefore, the ideal source and number of MSCs needed to promote optimal tissue regeneration is yet under investigation.

Table 89.1 Differences among bone marrow-derived MSC, adipose-derived MSC, and allogenic MSC

Bone marrow-derived MSCs (BM-MSC)	Adipose-derived MSCs (AD-MSC)
Advantages	**Advantages**
Harvest is easy to perform	Cellular expansion is not required to improve efficacy
Minimal processing is required	May be used in conjunction with PRP for a combined effect·
BM-MSC are hypoimmunogenic; therefore, immunosuppression is not required for allogenic transplantations	AD-MSC are hypoimmunogenic; therefore, immunosuppression is not required for allogenic transplantations
Disadvantages	**Disadvantages**
Greater lineage differentiation potential	Harvest is technically challenging and requires tumescent liposuction and other additional equipment
Cellular expansion prohibited under US FDA regulation. If manipulated (e.g., through in vitro expansion), the cells cannot be injected back into the donor	Requires a lengthy multistep processing, including enzymatic digestion, washes, and cellular cultures
May differentiate uncontrollably into an undesired lineage (i.e., fibroblasts to cause scarring)	The amount of adipose tissue that can be harvested and processed is contingent upon the patient's size and body fat content
	Lower differentiation potential into desired osteogenic and chondrogenic lineages, instead, preferentially differentiating into adipocytes
Allogenic MSC	
Advantages	
Potentially painful harvesting procedure is eliminated	
Off-the-shelf" pre-expanded and preprocessed cell lines are readily for use with a constant supply	
Hypoimmunogenic	
No preferential cellular lineage differentiations	
Decreased processing requirements reduce infection risks	
Mesenchyme precursor cells (MPC-06-ID) by Mesoblast Ltd. shows promising results during intradiscal clinical trials	
Disadvantages	
Still in clinical trial (phase 3)	

Allogeneic bone marrow stromal cells have been the most extensively studied. Animal studies demonstrated survival and replication up to 48 weeks after transplantation and restored disc height and proteoglycan content, 6 months after single injection. Restored disc height and improved symptomatology following allogeneic stem cells injection have also been reported in humans. Currently, "off-the-shelf" bone marrow-derived allogenic stem cells are undergoing phase 3 clinical trial (Mesoblast Ltd.) and are showing promising potential in the treatment of degenerative disc disease (Table 89.2).

Growth Factors

MSCs induce direct cellular interactions and signaling via growth factors and anti-inflammatory cytokine secretions to activate endogenous progenitor cells in the pathologic tissues that were previously dormant. These growth factors contribute significantly to the angiogenic and healing properties of stem cells. This results in tissue regeneration with reduction in pain and inflammatory response at the affected area. Some of the growth factor examples are the following:

- Transforming growth factor-β1 (TGF-β1)
  - Regulate cellular proliferation and differentiation
- Vascular endothelial growth factor (VEGF)
  - Stimulate neovascularization
- Placental growth factor (PGF)
  - Promote angiogenesis
- Hepatocyte growth factor (HGF)
  - Responsible to wound healing and organ regeneration
- Stromal-derived factor-1 (SDF-1)
  - Homing factor: recruit and retain progenitor cells to the injury site via chemotaxis
- Fibroblast growth factor (FGF-2)
  - Facilitate angiogenesis and wound healing

Scaffolds

The biocompatible scaffolds serve as a three-dimensional interface to allow the MSCs to undergo proliferation, maturation, and matrix deposition. Examples of injectable scaffold materials are:

- Fibrin glue
- Platelet-rich plasma
- Hyaluronic acid
- Platelet lysate
- Collagen-rich extracellular matrix derived from dermis and small intestine submucosa

Table 89.2 Evidence for efficacy table

Reference	Study design	Size	Indication	MSC source	Outcomes
Degenerative joint disease					
Centeno (2008)	Case report	1	Knee OA	Bone marrow	Increased meniscus and cartilage volume on MRI Increased knee range of motion 95% pain reduction
Davatchi (2011)	Case series	4	Knee OA	Bone marrow	Mild improvement in pain and gait
Centeno (2011)	Case series	339	Multiple joints	Bone marrow	Most improvements seen in patients with knee OA
Richter (2013)	Case series	25	Foot/ankle chondral defect	Bone marrow	Improved pain and disability scores
Vagsness (2014)	Randomized clinical trial	55	Knee meniscal tear	Bone marrow	Partial meniscectomy with MSC injection group experienced a greater pain reduction than meniscectomy alone group
Centeno (2014)	Case series	196	Hip OA	Bone marrow	Most improvement seen in ≤55 age group. 6.4 points improvement on Oxford Hip Scale (OHS) 1.2-point reduction on numeric pain scale (NPS)
Vega (2015)	Randomized clinical trial	30	Knee OA	Bone marrow	MSC group reported significant pain and functional mobility improvement compared to hyaluronic acid group
Koh (2016)	Randomized clinical trial	80	Knee OA	Adipose	Mircrofracture with MSC and fibrin glue group demonstrated improved pain and functional mobility scores than microfracture alone group
Tendinopathy					
Connell (2009)	Case Series	12	Lateral epicondylopathy	Dermal fibroblast	Improved in clinical scores and ultrasound imaging
Clark (2011)	Randomized clinical trial	46	Chronic patella tendinopathy	Tenocyte-like cells	Faster healing response seen on ultrasound imaging Improved clinical response
Hernigou (2014)	Case controlled	45	Rotator cuff tear	Bone marrow	MSC group had a low rate or re-injury compared to control following rotator cuff repair
Centeno (2015)	Case series	102	Shoulder OA and rotator cuff tear	Bone marrow	MSC therapy improved upper extremity function and reduced shoulder pain
Degenerative disc disease					
Meisel (2007)	Randomized clinical Trial	28	Degenerative disc disease	Disc chondrocytes	Improved pain and disability scores sustained at 24 months
Yoshikawa (2010)	Case series	2	Degenerative disc disease	Bone marrow	Clinical and radiographic improvements recorded
Orozco (2011)	Case series	10	Degenerative disc disease	Bone marrow	Increased disc hydration on MRI. Rapid pain and disability improvement
Pang (2014)	Case series	2	Degenerative disc disease	Umbilical cord blood	Improved pain and disability scores
Mochida (2015)	Case series	9	Degenerative disc disease	Autologous nucleus pulposus with marrow-derived MSC	Minimally efficacious
Pettine (2016)	Case series	26	Degenerative disc disease	Bone marrow	Improved pain and disability scores sustained over 24 months
Neuropathic pain					
Vickers (2014)	Case series	10	Trigeminal neuralgia	Adipose	Improved pain and disability scores sustained at 6 months
Venturi (2015)	Case series	15	Pudendal neuralgia	Adipose	Improved pain and disability scores sustained at 12 months

Mechanical Stimulation

Studies have shown that in addition to cytokines and growth factors, mechanical forces also play an important role in MSCs differentiation such as:

- Weight-bearing exercises provide mechanical stimulation for axial bone and muscle mass development and maintenance.
- Under tensile stress (stretching), MSCs have shown to preferentially undergo osteogenesis and tenogenesis in vitro.
- Similarly, hydrostatic pressure, compressive loading, and hypoxic environment induce chondrogenesis.

These findings translated to using fixators and continuous passive machines to enhance bone and knee joint healing, respectively. While the importance of mechanical stimulation

is appreciated in regulating MSCs, incorporating this knowledge into regenerative and rehabilitation medicine protocols remains a complex issue.

Uses and Indications

Musculoskeletal conditions that are commonly treated with stem cell technology include:

- Knee/hip/ankle osteoarthritis and rheumatoid arthritis
- Tendinopathy
- Partial ligament or meniscal tear
- Hip or shoulder labral tear
- Degenerative intervertebral disc disease

Despite broad regenerative therapy indications, physicians must comply with the regulations set forth by the US Food and Drug Administration (FDA). The FDA mandates that in order for cell and tissue product to be exempt from pre-marketing approval, the biologics must meet the following criteria:

- Minimally manipulated. Minimal manipulation is defined as:
  - Structural tissues: processing that does not alter the original relevant characteristics of the tissue relating to the tissue's utility for reconstruction, repair, or replacement.
  - Cells or nonstructural tissues: processing that does not alter the relevant biological characteristics of cells or tissues. For example, the use of collagenase is considered more than "minimally manipulated" by current good manufacturing practice requirements. However, nonenzymatic isolation methods using mechanical protocols are deemed less than minimally manipulated.
- Homologous use.
- Cell numbers cannot be expanded.
- Not mixed with other substances for the purpose of preservation, sterilization, or storage.
- Does not exert systemic or metabolic influence.
- Limited to autologous or administering in first- or second-degree relatives.

Contraindications to stem cell therapy in musculoskeletal conditions:

- Complete ligament or tendon rupture
- Intra-articular loose body
- Active local or systemic infections
- Uncontrolled cardiovascular disease
- Bleeding disorders

Stem Cells Source and Harvesting Technique #1: Bone Marrow Aspirate Concentrate (BMAC)

- Position the patient prone on a procedure table, and target the marrow extraction sites around the posterior superior iliac spine (PSIS) as bone marrow can be easily aspirated from this thick portion of the iliac crest.
- Prepare and drape the intended site using strict sterile technique.
- Use ample amount of local anesthetics to anesthetize the skin (e.g., 10–15 cc of 1% lidocaine or 0.25% ropivacaine). Lidocaine is cytotoxic; therefore, it must not come in contact with the bone marrow aspirate.
- Ensure sufficient time and anesthetics are allocated to allow the skin and soft tissue to be effectively anesthetized.
- Unless otherwise indicated by the instructions of the kit, fill the draw syringes with 1000 units of heparin per cc of aspirant to be drawn.
- Fill a 30 cc and a 5 cc syringe with 30,000 units and 5000 of heparin per syringe, respectively.
- Under direct ultrasound or fluoroscopic guidance, insert the needle until the tip is in direct contact with the bone cortex and then sequentially advance the guidewire, stylet/trocar. Maintain a forward pressure while turning the trocar clock- and counterclockwise until 5–10 mm of the trocar tip is seated in the cortex.
- Gently twist the trocar to ensure the trocar-bone cortex is securely snug.
- Remove the stylet from the trocar, and recheck if firmly implanted in the cortex by performing a second wiggle test. If not, then advance the trocar until firm, not exceeding approximately 1 cm in depth.
- Secure the 5 cc syringe with the 5000 units of heparin to the trocar, and inject approximately 500–750 units of heparin into the marrow space immediately on entry into the cortex. It is crucial to prevent the marrow sample from clotting as this renders MSC unusable. Repeat this step for each target aspiration site.
- Remove the 5 cc syringe and attach the draw syringe to the trocar. Match the aspiration speed to the patient's tolerance.
- Drawing a large volume (over 20 cc) from a single bone site reduces MSC yield. We recommend drawing small volumes (5–15 cc) from many sites to increase yield.
- Gently agitate the syringe to mix the heparin and the initial bone marrow aspirate. Once thoroughly mixed, only draw 5–15 cc of bone mineral aspirate per site.
- Move the trocar tip to a new cortex for aspiration without retrieving the trocar from the skin.

- Draw volumes are decided based on the patient weight and the dimension of the treatment area. Use the following guidelines:
  - Females <105 pounds: do not draw more than 50 cc.
  - Female patients between 105 and 120 pounds: the upper acceptable volume limit is 60 cc.
  - Patients of either sex between 120 and 180 pounds: the upper acceptable volume limit is 90 cc.
  - Male patients >180 pounds: up to 120 cc can be collected.

BMAC Preparation and MSC Quantification

Bone marrow aspirate concentrate can be processed with various automated commercial kits or prepared manually inside a biosafety cabinet. Regardless of which method is used, the end goal is to isolate the buffy coat. At this time, there are limited data comparing the MSC outputs of various concentration devices.

Quantification can be accomplished by using the following methods:

- Flow cytometry – This technique uses fluorescent antibodies to bind the specific cell surface markers. Surface markers stained with various colored antibodies intact differently with a laser beam, which enables the amount of BMCs to be measured. This method requires expertise to interpret the results, and it is often cost inhibitive for routine clinic uses.
- Colony-forming unit (CFU) assay – BMC is cultured and incubated until colonies of MSCs form. These MSCs are counted. This method of estimating MSC in BMC is mostly used in the lab and not at the bedside.
- Total nucleated cell count (TNC) – A manual hemocytometer or a commercial automated counting system can be used to indirectly estimate the MSC amount in a BMC sample.
- A higher CFU or TNC is associated with better clinical outcome.

Stem Cell Source and Harvesting Technique #2: Adipose Tissue SCF via Lipoaspiration

Tumescent Fluid Preparation and Dosing

Tumescent mixture is the standard anesthetic solution used for liposuction procedures. The solution is infiltrated into the subcutaneous tissues to provide local anesthesia during the procedure.

The tumescent fluid amount required is determined by:

1. The patient's weight: the maximum lidocaine dosage is 4.5 mg/kg or 7 mg/kg when combined with epinephrine. The standard recommended tumescent lidocaine is 3.5 mg/kg (27).
2. The amount of adipose to be harvested.
   - For small amounts of adipose tissue (60–120 cc), 0.1% tumescent solution may be used. 0.1% tumescent solution can be prepared in a 1000 ml bag of 0.9% sodium chloride by mixing the following:
     - 50 ml 2% lidocaine
     - 1 ml 1:1000 epinephrine
     - 10 ml 8.4% sodium bicarbonate
   - For larger amounts of adipose tissue (>120 cc), 0.05% tumescent solution may be used. Similarly, 0.05% tumescent solution can be prepared in a 1000 ml bag of 0.9% sodium chloride by mixing the following:
     - 25 ml 2% lidocaine
     - 1 ml 1:1000 epinephrine
     - 8 ml 8.4% sodium bicarbonate
3. The tumescent solution should be mixed on the same day as the procedure, and the epinephrine should be added immediately prior to use. The bag should be clearly identified and dated.

Infiltration of Tumescent Anesthetic Fluid

- The patient is positioned supine for abdominal adipose harvesting and lateral decubitus for flank or hip adipose harvesting.
- Vital sign monitoring is placed.
- Drape and clean the lipoaspiration site while adhering to strict sterile technique.
- If harvesting adipose tissue from the abdomen, the intended port site(s) should be placed in line with the bilateral anterior axillary line, at the level of the anterior iliac spine. If the second port site is needed, it should be staggered to the first site.
- Anesthetize the skin over the marked target port sites.
- Use a number 11 scalpel to make a 3 mm incision.
- Insert a 14G garden spray infiltration needle to saturate area of lipoaspiration with the tumescent fluid.
- The tumescent fluid IV bag maybe pressurized with a pressure bag in conjunction with gravitational assist. Another method is to deliver the tumescent fluid via a 60 cc syringe.
- Regardless of the delivery method, tumescent fluid infiltration should be deposited slowly and evenly throughout the subcutaneous tissue.

- Maintain the infiltration needle parallel with the abdominal wall to prevent any unintentional transabdominal or peritoneal perforation injury.
- The skin over the abdomen may appear firm with demarcating blanching following sufficient tumescent fluid with epinephrine infiltration. At this time, the physician may begin lipoaspiration.

Lipoaspiration Technique

- Attach a Mercedes Lipo Cannula (3 mm × 25 cm) to a 60 cc Toomey syringe.
- After the needle is held subcutaneously, the syringe plunger is pulled back to create roughly 18 inches of Hg vacuum. A snap lock or Johnnie snap will keep the plunger in the retracted position to maintain a negative pressure inside the syringe.
- Maneuver the needle back and forth in a fan-shaped fashion throughout the subcutaneous tissue as the negative pressure pulls the lipoaspirate into the syringe.
- The physician should place the non-dominant hand on the patient's abdomen to ensure that the needle tip remains at the safe distance from the superficial skin and the needle does not extend beyond the intended treatment area.
- We recommend sequentially aspirate the deeper layers followed by more superficial regions.
- Avoid removing excess adipose tissue from any one area as this results in skin dimpling.
- Continue to suction the aspirate until the syringe is full.
- Place the syringe right on a rack to allow the fat to rise above the supernatant fluid.
- Discard any supernatant fluid into a sterile stainless steel basin, and repeat this step until the desired volume of fat has been obtained.
- Cap the syringe before transporting to the processing area.

Post-Lipoaspiration Procedures

- Gently express any excess tumescent fluid through the port site(s).
- Approximate the skin edges, and secure the puncture wound with steri-strips before dressing the area with an ABD pad.
- Following an abdominal adipose tissue harvest, an elastic abdominal binder will generally be sufficient to provide comfort, minimize bruising, and improve skin aesthetics. Similarly, a compression body suit may be useful when adipose tissue is extracted from thigh or hip subcutaneously.
- The patient should wear the compression device continuously for the first 72 hours followed by minimal interruptions for the next 3–4 days.
- Continue to monitor the patient's vital signs until the patient is stable to be discharged.

Adipose SVF Processing

- SVF from adipose tissue can be safely processed either by mechanical or enzymatic means.
- Mechanical separation method is preferred when extracting SVF from a lower adipose tissue amount because mechanical processing is more economical and requires a shorter processing turnaround time. However, the mechanical method yields fewer progenitor cells and a higher mononuclear cell count.
- Enzymatic processing is expensive, but it has been shown to provide a more consistent and reliable way of breaking down extracellular matrix. The authors advocate the enzymatic isolation.
  - Enzymatic isolation of the SVF
    - All specimens should be clearly marked with patient identifiers.
    - We recommend that all tissue handling outside of the sterile procedure suite occur under a Class 100 HEPA-Filtered Laminar Flow Biological Cabinet using aseptic technique.
    - The basic steps universally utilized to isolate adipose stem cells involve a cell wash, collagenase digestion, followed by centrifugal separation and filtration to isolate the single cell stromal vascular fraction from the primary adipocytes.
    - There are several commercially available, single-use kits that offer proprietary formulas and unique prepackaged digestive enzymes.
    - SVF is resuspended in a carrier solution for final treatment. The carrier solutions include autologous platelet-rich plasma (PRP) and preservative-free normal saline. Autologous PRP is the authors' recommended scaffold solution for musculoskeletal, intrathecal, or intravascular therapeutic applications. The total resuspension volume may range from 2 to 10 cc depending on the site of treatment.

Uses and Indications

Intradiscal Application

Preprocedure Considerations

- Other sources, such as facet- and/or sacroiliac joint-mediated low back pain, must be ruled out.

- Magnetic resonance imaging (MRI) must demonstrate mild disc degeneration: less than 50% disc height loss and no more than grade 1 listhesis at the targeted level.
- As previously stated, allogenic stem cells are demonstrating excellent clinical outcomes during phase 3 clinical trial at the time of this chapter writing. Mesoblast may soon be available for clinical use.

Technical Aspects

- Posterolateral intradiscal approach
  - Patient is placed in a prone position on the fluoroscopy table.
  - The skin over the lumbar area is carefully cleaned and draped using strict sterile techniques.
  - AP view of the lumbar spine is centered on the target disc with the vertebral endplates clearly focused and aligned.
  - C-arm is obliqued contralaterally to the uninvolved side to produce the trajectory view.
  - Safe needle advancement is performed under direct fluoroscopic visualization using lateral views.
  - We recommend using the two-needle technique (a smaller gauge needle is inserted through the larger gauge needle) so that the needle that pierces the annulus does not first contact the skin to minimize infection risk.
  - Needle(s) is (are) advanced into Kambin's triangle under intermittent fluoroscopic guidance.
  - Do not use contrast dye during this procedure because contrast material may impede with cell regenerative functions.
  - Cellular injectate is slowly deposited with the needle tip properly positioned inside the disc.
  - A minute amount (0.5 cc) of preservative-free normal saline can be flushed into the disc before withdrawing the needle. This minimizes the risk of stem cells tracking into the epidural space during needle retraction.
  - Instruct the patient to avoid rigorous activities for 1–2 days as a general precaution.
  - Apply sterile dressing to needle entry site.

Intra-articular Application

Lumbar Facet Joint

- The patient lies prone with a pillow under the abdomen for comfort.
- Direct the C-arm beam parallel to the facet joint to allow direct visualization of the open facet joint space.
- Mark the skin where the beam is projected, and clean the area using strict sterile technique.
- Anesthetize the skin and superficial soft tissues using a 27G needle and 1–2% lidocaine.
- Insert a 21G, 3.5 inch spinal needle under fluoroscopic visualization.
- 0.1–0.2 ml nonionic contrast material can be injected into the joint space to confirm intra-articular needle placement.
- Slowly inject 0.5 ml of stem cell suspension with low pressure to prevent capsule rupture.

Knee Joint Injection

- Place the patient supine with the treatment knee slightly flexed (place a pillow or a bolster under the treatment knee for support), and close to the side of the table where the physician will be standing.
- Place the ultrasound probe in long axis to bring the patella, the femur, and the joint recess in view.
- While keeping the knee joint recess in the center of the screen, turn the probe 180° to examine the anterior knee in the short-axis view.
- Ensure the joint recess can be clearly visualized.
- The needle entry point and depth can be determined in the short-axis view.
- Clean the skin using strict sterile technique and mark the skin.
- A sterile sheath-covered ultrasound probe is reapplied to obtain short-axis view.
- A 22G, 1.5 inch needle is advanced under direct in-plane visualization toward the joint recess.
- Aspirate to monitor for blood before injecting.
- Once the needle tip enters the joint recess, the injectate should be advanced into the joint space without resistance.
- Inject the stem cell suspension while moving the needle in a fenestrating-fan fashion.
- Carefully withdraw the needle and apply a sterile dressing.

Hip Joint Injection

- Place the patient supine with the treatment hip close to the side of the table where the physician will be standing.
- Place the ultrasound probe in long axis to the femoral neck to identify the femoral head, neck, and anterior recess. Because of the femoral neck angle, the probe orientation will be in the oblique sagittal plane.
- Hip vasculatures, medial to the hip joint, can be further delineated using the power Doppler function. The acetabular labrum appears hyperechoic and extends from the margins of the acetabulum.

- Clean the skin using strict sterile technique, and mark the skin 2–3 cm lateral to the vascular bundle in line with the femoral neck.
- Anesthetize the skin and superficial soft tissues using a 27G needle and 1–2% lidocaine.
- A sterile sheath-covered ultrasound probe is reapplied to obtain view.
- A 22G, 3.5 inch needle is advanced under direct visualization toward the femoral neck.
- Aspirate to monitor for blood before injecting.
- Once the needle tip is inside the hip capsule, inject the stem cell suspension while moving the needle in a fenestrating-fan fashion.
- Carefully withdraw the needle and apply a sterile dressing.

Glenohumeral Joint Injection

- The patient is seated with the arm in internal rotation to rest the hand on the ipsilateral hip. This position facilitates the opening of the posterior joint space.
- Place the transducer parallel the scapular spine, and move inferiorly until infraspinatus and teres minor are identified. The probe is then turned long axis to examine the posterior labrum (hyperechoic triangularly shaped).
- Lower frequency may provide a better visualization of deeper structures in large or athletic shoulders.
- Identify the position of the labrum in the middle of the computer screen.
- Mark the needle entry point with a surgical marker.
- Carefully clean the skin with chlorhexidine or betadine or 70% isopropyl alcohol using sterile technique.
- 3–5 cc of 1–2% lidocaine is used to anesthetize the skin and superficial soft tissues.
- Position the sterile sheath-covered ultrasound probe to center the image of the lesion on the computer screen.
- A 22G, 3.5 inch needle is placed 1 cm lateral to the lateral edge of the transducer and advanced using "in-plane technique" medially toward the glenohumeral joint space.
- Maintain constant visualization of the needle tip to avoid penetrating the labrum.
- Slowly deliver the injectate while moving the needle in a fenestrating-fan fashion.
- Carefully withdraw the needle and apply a sterile dressing.

Tendinous Application

Supraspinatus and Subscapularis Injection: Anterior Approach to Rotator Cuff Interval

- Supraspinatus and subscapularis tendinopathies are best addressed using this technique.
- Supraspinatus: the patient is seated with the hand placed on the ipsilateral hip area and with the elbow pointed posteriorly while maintaining shoulder external rotation (modified Crass position).
- Subscapularis: the patient is seated with forearm in supination and shoulder external rotation.
- The ultrasound probe is placed in short axis on the anterior shoulder just superior to the greater and lesser tuberosity.
- The biceps tendon is visualized between the supraspinatus and subscapularis tendons.
- Identify the lesion, and position the image of the lesion in the middle of the computer screen. Tears are most often found in the distal 1/4 of the tendon in the transverse direction.
- Mark the needle entry point with a surgical marker.
- Carefully clean the skin with chlorhexidine or betadine or 70% isopropyl alcohol using sterile technique.
- 3–5 cc of 1–2% lidocaine is used to anesthetize the skin and superficial soft tissues.
- Position the sterile sheath-covered ultrasound probe to center the image of the lesion on the computer screen.
- A 20 or 22 gauge, 3.5 inch needle is advanced using "in-plane technique" toward the space just lateral to the coracoid process at the humeral head.
- The needle is advanced into the rotator cuff interval toward the supraspinatus tendon for supraspinatus injection.
- The area caudal to the coracoid process is the target for the subscapularis injection.
- Slowly inject the stem cell suspension while moving the needle in a fenestrating-fan fashion to immerse the damaged area.
- Carefully withdraw the needle and apply a sterile dressing.

Infraspinatus and Teres Minor Injection: Posterior Approach

- The patient is seated with the arm by the side and the hand resting on the ipsilateral thigh. This position facilitates the opening of the posterior joint space.
- Place the transducer angled obliquely and superiorly toward the humeral head and parallel to the scapular spine inferiorly.
- Identify the lesion, and position the image of the lesion in the middle of the computer screen.
- Mark the needle entry point with a surgical marker.
- Carefully clean the skin with chlorhexidine or betadine or 70% isopropyl alcohol using sterile technique.
- 3–5 cc of 1–2% lidocaine is used to anesthetize the skin and superficial soft tissues.

- Position the sterile sheath-covered ultrasound probe to center the image of the lesion on the computer screen.
- A 20 or 22G, 3.5 inch needle with an approximately 30-degree tip curvature is advanced using "in-plane technique" medially toward the glenohumeral joint space.
- Slowly inject the stem cell suspension while moving the needle in a fenestrating-fan fashion to immerse the damaged area.
- Carefully withdraw the needle and apply a sterile dressing.

Clinical Pearls and Pitfalls

- Universal precautions should be followed strictly by all personnel involved in handling patient specimens.
- When permissible, medications such as statins, corticosteroids, NSAIDs, and ACE inhibitors should be held for at least 2–4 weeks following MSC therapy because of their MSC inhibitory effects.
- Continuous monitoring of vital signs with airway resuscitative equipment and crash cart in reach is recommended as there have been transient rise in blood pressure, headache, and vasovagal response following parenteral administration of regenerative injectate.
- Refrain from using antibiotics and radio-opaque material, as they have been reported to be cytotoxic.
- Do not use needles less than 22 gauge to introduce the stem cell matrix into the patient. Smaller needles will affect the structural integrity of stem cells.
- Select appropriate injectate volume to match the volume which the target body part is able to accommodate.
- As with any injection therapy, correctly identifying the pain generator will render highest chance for a successful treatment.
- The recommended site to harvest bone MSC is the iliac crest.
- Provide ample local anesthesia from the skin and deep tissues down to the periosteum to prevent intense pain and poor patient experience.
- Only use 0.25% or less of ropivacaine. All other local anesthetics are cytotoxic regardless of anesthetics concentration.
- Allow sufficient time for local anesthesia to provide coverage.
- When collecting bone marrow, use multiple smaller syringes to optimize a higher specimen yield.
- Heparin is preferred over anticoagulant citrate dextrose (ACD) to prevent clotting of bone marrow aspirate inside the draw syringe (i.e., minimize the risk of cell count loss inside the syringe). Heparin must also be used at the cannulation site on the bone which will also prevent clotting.
- Recommended rehabilitation protocol:
  - 0–2 weeks: isometric exercises without range of motion.
  - 3–5 weeks: range of motion exercises, isotonic exercises with low-level resistance.
  - >6 weeks: eccentric exercises can be added as tolerated. Full physical activity can be resumed at 8–10 weeks.

Recommended Reading

1. Centeno CJ, Busse D, Kisiday J, Keohan C, Freeman M, Karli D. Increased knee cartilage volume in degenerative joint disease using percutaneously implanted, autologous mesenchymal stem cells. Pain Physician. 2008;11(3):343–53.
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