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MSC (MESENCHYMAL STEM CELL): NEW HORIZON IN HEPATECTOMY

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Abstract: Mesenchymal stem cells (MSCs) are stromal cells that have the ability to self-renew and also exhibit multilineage differentiation. MSCs can be isolated from a variety of tissues, such as umbilical cord, endometrial polyps, menses blood, bone marrow, adipose tissue, etc. This is because the ease of harvest and quantity obtained make these sources most practical for experimental and possible clinical applications. Recently, MSCs have been found in new sources, such as menstrual blood and endometrium. The multipotent properties of MSCs make them an attractive choice for possible development of clinical applications. Future studies should explore the role of MSCs in differentiation, transplantation, and immune response in various diseases. Mesenchymal stem cells can also regenerate and repair damaged liver cells. In mouse models of liver fibrosis, mesenchymal stem cells delivered to the liver were shown to improve liver function by reducing inflammation and necrosis and inducing hepatocyte regeneration.

Keywords: MSC, Bone Marrow, Amniotic Fluids

Introduction: Mesenchymal stem cells (MSCs) also known as mesenchymal stromal cells or medicinal signaling cells are multipotent stromal cells that can differentiate into a variety of cell types, including osteoblasts (bone cells), chondrocytes (cartilage cells), myocytes (muscle cells) and adipocytes (fat cells which give rise to marrow adipose tissue).

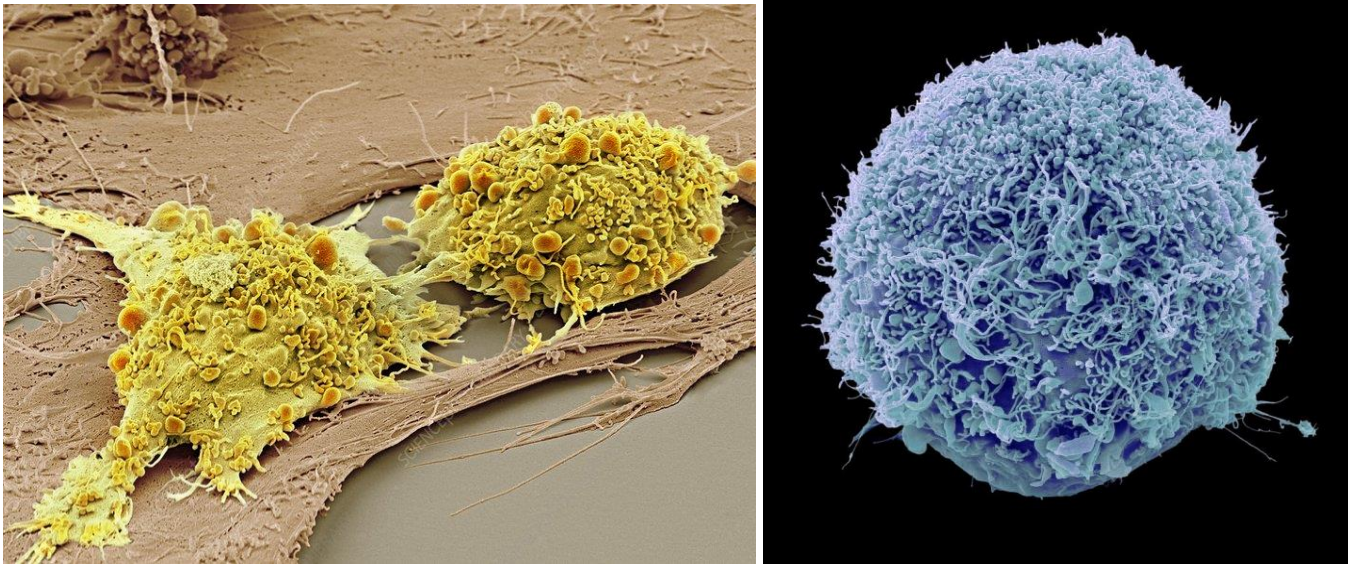


Figure-1: Mesenchymal stem cell

Structure:

Definition: While the terms mesenchymal stem cell (MSC) and marrow stromal cell have been used interchangeably for many years, neither term is sufficiently descriptive:

- Mesenchyme is embryonic connective tissue that is derived from the mesoderm and that differentiates into hematopoietic and connective tissue, whereas MSCs do not differentiate into hematopoietic cells.
- Stromal cells are connective tissue cells that form the supportive structure in which the functional cells of the tissue reside. While this is an accurate description for one function of MSCs, the term fails to convey the relatively recently discovered roles of MSCs in the repair of tissue.^[1]
- The term encompasses multipotent cells derived from other non-marrow tissues, such as placenta, umbilical cord blood, adipose tissue, adult muscle, corneal stroma or the dental pulp of deciduous (baby) teeth. The cells do not have the capacity to reconstitute an entire organ.

Morphology

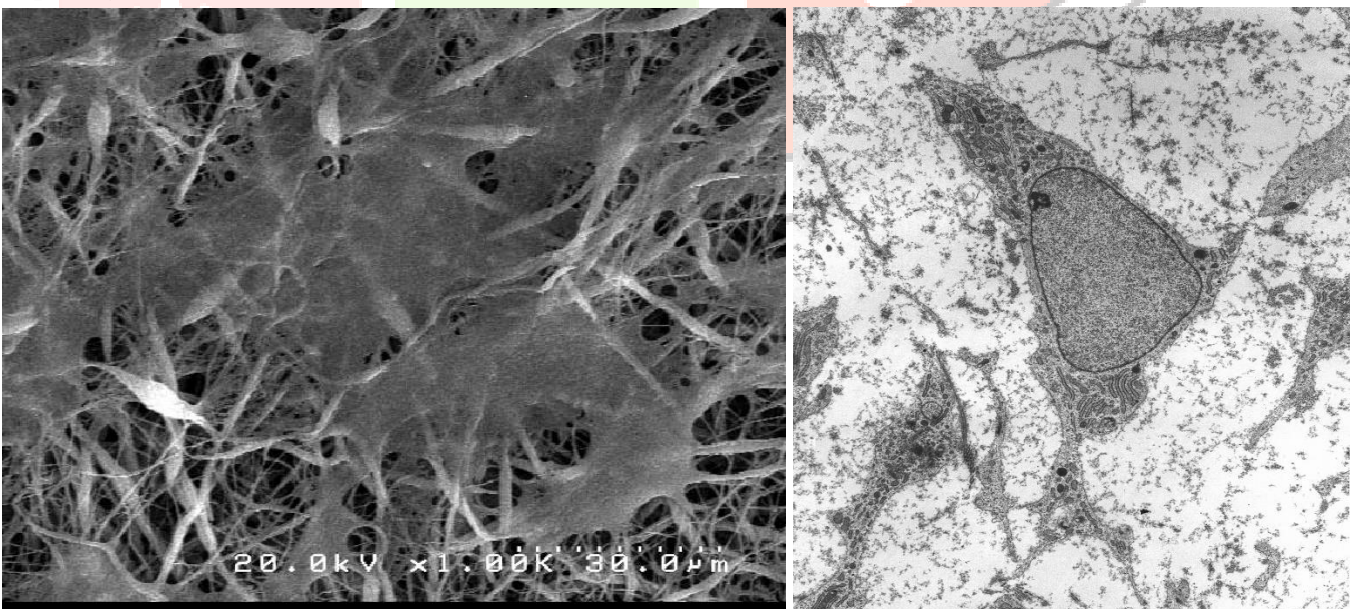


Figure-2: Human bone marrow derived Mesenchyme stem cell showing fibroblast-like morphology seen under phase contrast microscope

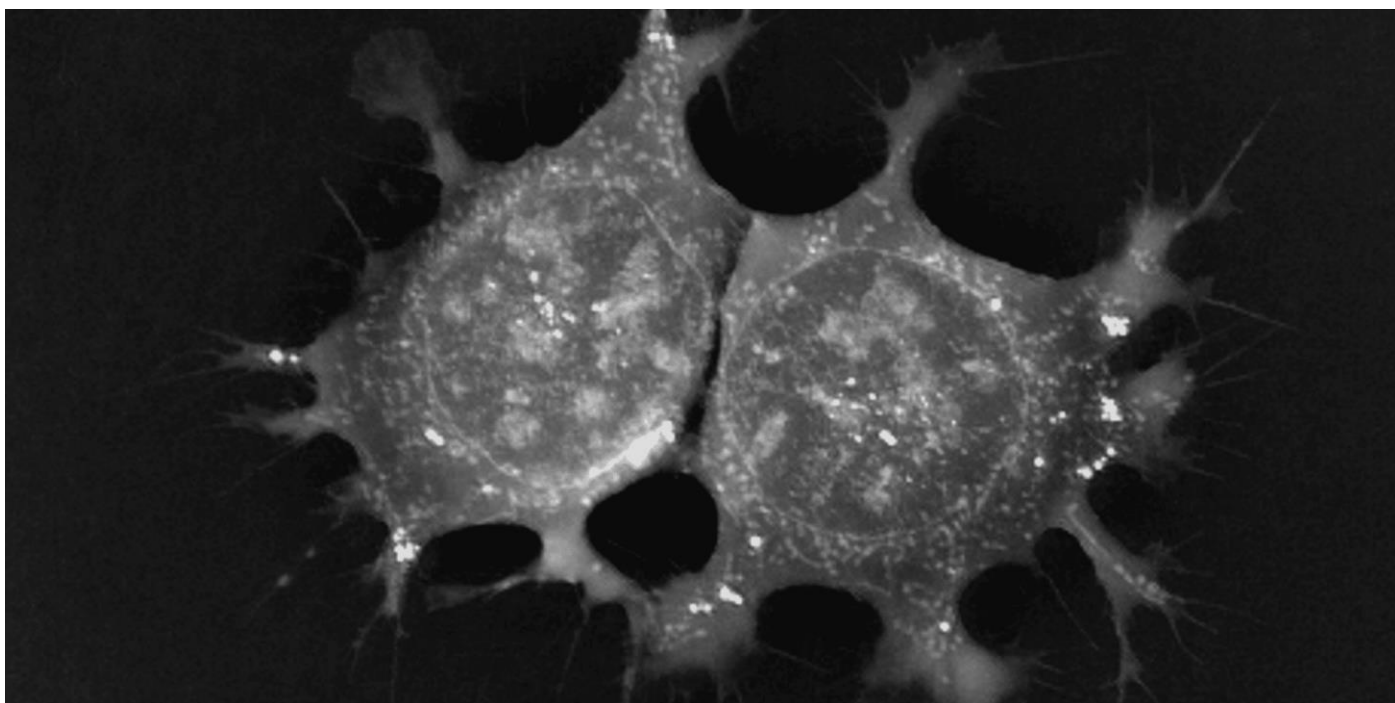


Figure-3: An example of human mesenchymal stem cells imaged with a live cell imaging microscope

Mesenchymal stem cells are characterized morphologically by a small cell body with a few cell processes that are long and thin. The cell body contains a large, round nucleus with a prominent nucleolus, which is surrounded by finely dispersed chromatin particles, giving the nucleus a clear appearance. The remainder of the cell body contains a small amount of Golgi apparatus, rough endoplasmic reticulum, mitochondria and polyribosomes. The cells, which are long and thin, are widely dispersed and the adjacent extracellular matrix is populated by a few reticular fibrils but is devoid of the other types of collagen fibrils. These distinctive morphological features of mesenchymal stem cells can be visualized label-free using live cell imaging.^[2]

Location:

Bone marrow: Bone marrow was the original source of MSCs, and still is the most frequently utilized. These bone marrow stem cells do not contribute to the formation of blood cells and so do not express the hematopoietic stem cell marker CD34. They are sometimes referred to as *bone marrow stromal stem cells*.

Cord cells: The youngest and most primitive MSCs may be obtained from umbilical cord tissue, namely Wharton's jelly and the umbilical cord blood. However MSCs are found in much higher concentration in the Wharton's jelly compared to cord blood, which is a rich source of hematopoietic stem cells. The umbilical cord is available after a birth. It is normally discarded and poses no risk for collection. These MSCs may prove to be a useful source of MSCs for clinical applications due to their primitive properties and fast growth rate and these have several advantages over bone marrow-derived MSCs. Adipose tissue-derived MSCs (AdMSCs), in addition to being easier and safer to isolate than bone marrow-derived MSCs, can be obtained in larger quantities.^[3]

Molar cells: The developing tooth bud of the mandibular third molar is a rich source of MSCs. While they are described as multipotent, it is possible that they are pluripotent. They eventually form enamel, dentin, blood vessels, dental pulp and nervous tissues. These stem cells are capable of differentiating into chondrocytes, cardiomyocytes, melanocytes, and hepatocyte-like cells *in vitro*.

Amniotic fluid: Stem cells are present in amniotic fluid. As many as 1 in 100 cells collected during amniocentesis are pluripotent mesenchymal stem cells.

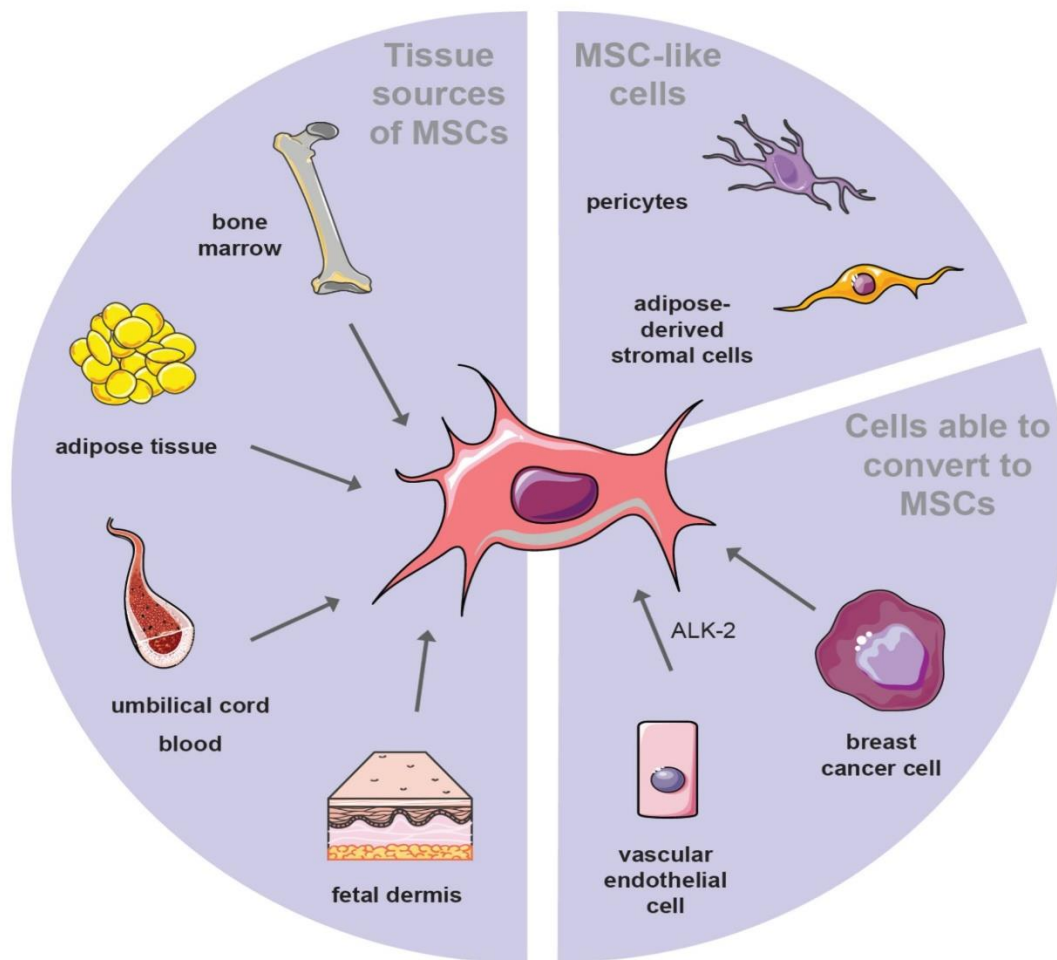


Figure-4: Sources of MSC (Mesenchymal Stem Cell)

Function:

Differentiation capacity: MSCs have a great capacity for self-renewal while maintaining their multipotency. Recent work suggests that β -catenin, via regulation of EZH2, is a central molecule in maintaining the "stemness" of MSC's. The standard test to confirm multipotency is differentiation of the cells into osteoblasts, adipocytes and chondrocytes as well as myocytes.^[4]

MSCs have been seen to even differentiate into neuron-like cells, but doubt remains about whether the MSC-derived neurons are functional. The degree to which the culture will differentiate varies among individuals and how differentiation is induced, e.g., chemical vs. mechanical; and it is not clear whether this variation is due to a different amount of "true" progenitor cells in the culture or variable differentiation capacities of individuals' progenitors. The capacity of cells to proliferate and differentiate is known to decrease with the age of the donor, as well as the time in culture. Likewise, whether this is due to a decrease in the number of MSCs or a change to the existing MSCs is not known.

Immunomodulatory effects: MSCs have an effect on innate and specific immune cells, and research has shown an ability to suppress tumor growth. MSCs produce many immunomodulatory molecules including prostaglandin E2 (PGE2), nitric oxide, indoleamine 2,3-dioxygenase (IDO), interleukin 6 (IL-6), and other surface markers such as FasL, PD-L1 and PD-L2. MSCs have an effect on macrophages, neutrophils, NK cells, mast cells and dendritic cells in innate immunity. MSCs are able to migrate to the site of injury, where they polarize through PGE2 macrophages in M2 phenotype which is characterized by an anti-inflammatory effect. Further, PGE2 inhibits the ability of mast cells to degranulate and produce TNF- α . Proliferation and cytotoxic activity of NK cells is inhibited by PGE2 and IDO. MSCs also reduce the expression of NK cell receptors - NKG2D, NKp44 and NKp30. MSCs inhibit respiratory flare and apoptosis of neutrophils by production of cytokines IL-6 and IL-8. Differentiation and expression of dendritic cell surface markers is inhibited by IL-6 and PGE2 of MSCs. The immunosuppressive effects of MSC also depend on IL-10, but it is not certain whether they produce it alone, or only stimulate other cells to produce it.

MSC expresses the adhesion molecules VCAM-1 and ICAM-1, which allow T-lymphocytes to adhere to their surface. Then MSC can affect them by molecules which have a short half-life and their effect is in the immediate vicinity of the cell. These include nitric oxide, PGE2, HGF, and activation of receptor PD-1. MSCs reduce T cell proliferation between

G0 and G1 cell cycle phases and decrease the expression of IFN γ of Th1 cells while increasing the expression of IL-4 of Th2 cells. MSCs also inhibit the proliferation of B-lymphocytes between G0 and G1 cell cycle phases.

Antimicrobial properties: MSCs produce several antimicrobial peptides (AMPs) including human cathelicidin LL-37, β -defensins, lipocalin 2 and hepcidin. These peptides, together with the enzyme indoleamine 2,3-dioxygenase (IDO), are responsible for the broad-spectrum antibacterial activity of MSCs.

Clinical significance: Mesenchymal stem cells can be activated and mobilized if needed but their efficiency, in the case of muscle repair for example, is currently quite low. Further studies into the mechanisms of MSC action may provide avenues for increasing their capacity for tissue repair.^[5]

Autoimmune disease: Clinical studies investigating the efficacy of mesenchymal stem cells in treating diseases are in preliminary development, particularly for understanding autoimmune diseases, graft versus host disease, Crohn's disease, multiple sclerosis, systemic lupus erythematosus and systemic sclerosis. As of 2014, no high-quality clinical research provides evidence of efficacy, and numerous inconsistencies and problems exist in the research methods.

Other diseases: Many of the early clinical successes using intravenous transplantation came in systemic diseases such as graft versus host disease and sepsis. Direct injection or placement of cells into a site in need of repair may be the preferred method of treatment, as vascular delivery suffers from a "pulmonary first pass effect" where intravenous injected cells are sequestered in the lungs.

Detection: The International Society for Cellular Therapy (ISCT) has proposed a set of standards to define MSCs. A cell can be classified as an MSC if it shows plastic adherent properties under normal culture conditions and has a fibroblast-like morphology. In fact, some argue that MSCs and fibroblasts are functionally identical. Furthermore, MSCs can undergo osteogenic, adipogenic and chondrogenic differentiation *ex vivo*. The cultured MSCs also express on their surface CD73, CD90 and CD105, while lacking the expression of CD11b, CD14, CD19, CD34, CD45, CD79a and HLA-DR surface markers.

Research: The majority of modern culture techniques still take a colony-forming unit-fibroblasts (CFU-F) approach, where raw unpurified bone marrow or ficoll-purified bone marrow mononuclear cells are plated directly into cell culture plates or flasks. Mesenchymal stem cells, but not red blood cells or haematopoietic progenitors, are adherent to tissue culture plastic within 24 to 48 hours. However, at least one publication has identified a population of non-adherent MSCs that are not obtained by the direct-plating technique.

Other flow cytometry-based methods allow the sorting of bone marrow cells for specific surface markers, such as STRO-1. STRO-1+ cells are generally more homogenous and have higher rates of adherence and higher rates of proliferation, but the exact differences between STRO-1+ cells and MSCs are not clear. Methods of immunodepletion using such techniques as MACS have also been used in the negative selection of MSCs.

The supplementation of basal media with fetal bovine serum or human platelet lysate is common in MSC culture. Prior to the use of platelet lysates for MSC culture, the pathogen inactivation process is recommended to prevent pathogen transmission. New research titled Transplantation of human ESC-derived mesenchymal stem cell spheroids ameliorates spontaneous osteoarthritis in rhesus macaques various chemicals and methods including low level laser irradiation have been used to increase proliferation of stem cell.

Clinical uses of mesenchymal stem cells: Adult mesenchymal stem cells are being used by researchers in the fields of regenerative medicine and tissue engineering to artificially reconstruct human tissue which has been previously damaged. Mesenchymal stem cells are able to differentiate, or mature from a less specialized cell to a more specialized cell type, to replace damaged tissues in various organs.

Isolation of mesenchymal stem cells

Obtaining mesenchymal stem cells from the bone marrow: In the research process of expanding the therapeutic uses of mesenchymal stem cells, they are grown in laboratories or grown using medication to stimulate new cell growth within the human body. In mesenchymal stem cell therapy, most of the cells are extracted from the adult patient's bone marrow. Mesenchymal stem cells can be obtained via a procedure called bone marrow aspiration. A needle is inserted into the back of the patient's hip bone and cells are removed to be grown under controlled *in vitro* conditions in a lab. Over a course of two or three weeks, the cells will multiply and differentiate into specialized cells. The number of fully differentiated cells and their phenotype can be influenced in three ways. The first one is by varying the initial seed density in the culture medium. The second is by changing the conditions of the medium. The third is by the addition of additives such as proteins or growth hormones to the culture medium to promote growth. The mature cells are then harvested and injected back into the patient through local delivery or systemic infusion.^[6]

Isolation efficiency: Isolation of mesenchymal stem cells from the bone marrow requires an invasive procedure. Mesenchymal stem cells can also be isolated from birth-associated tissues such as the umbilical cord without the need for an invasive surgical procedure. Differences in isolation efficiency are attributed to the availability, condition, and age of the donor tissue. An issue related to culturing mesenchymal stem cells is the insufficient number of cells that can be produced. During long-term culture, mesenchymal stem cells age, lose their ability to differentiate, and have a higher chance to undergo malignant transformation.

Therapeutic properties: Mesenchymal stem cells possess many properties that are ideal for the treatment of inflammatory and degenerative diseases. They can differentiate into many cell types including bone, fat, and muscle which allow them to treat a large range of disorders. They possess natural abilities to detect changes in their environment, such as inflammation. They can then induce the release of bioactive agents and the formation of progenitor cells in response to these changes. Mesenchymal stem cells have also been shown to travel to sites of inflammation far from the injection site. Mesenchymal stem cells can be easily extracted through well-established procedures such as bone marrow aspiration. Also, transplanted mesenchymal stem cells pose little risk for rejection as they are derived from the patient's own tissue, so are genetically identical, however graft versus host disease is a possibility, where the cells change enough while outside the patient's body that the immune system recognizes them as foreign and can attempt to reject them. This can lead to symptoms such as itchiness, sensitive/raw skin and shedding or dry skin. [6]

Advantages over embryonic stem cells: Several different forms of stem cells have been identified and studied in the field of regenerative medicine. One of the most extensively studied stem cell types are embryonic stem cells, which possess many of the same therapeutic properties as mesenchymal stem cells, including the ability to self-regenerate and differentiate into a number of cell lineages. Their therapeutic abilities have been demonstrated in a number of studies of autoimmunity and neurodegeneration in animal models. However, their therapeutic potential has been largely limited by several key factors. Injected embryonic stem cells have been shown to increase the risk for tumor formation in the host patient. Also, the host's immune system may reject injected embryonic stem cells and thus eliminate their therapeutic effects. Finally, research has been largely limited due to the ethical issues that surround their controversial procurement from fertilized embryos.

Safety concerns: Human mesenchymal stem cell therapy is limited due to variation in individual response to treatment and the high number of cells needed for treatment. More long-term studies are needed to ensure the safety of mesenchymal stem cells. In previous studies which observed the safety of clinical mesenchymal stem cell use, no serious side effects were noted. However, there have been some cases where there were both improvement and toxicity inflicted on the targeted organ, as well as cases where treatment of mesenchymal stem cells did not show improvement of function at all. In addition, there is a risk of tumor genesis after stem cell transplantation due to the ability of stem cells to proliferate and resist apoptosis. Genetic mutations in stem cells as well as conditions at target tissue may result in formation of a cancerous tumor. Studies have shown that bone marrow mesenchymal stem cells can migrate to solid tumors and promote tumor growth in various cancer models through the secretion of proangiogenic factors. [7]

Treated disorders: Mesenchymal stem cells have been used to treat a variety of disorders including cardiovascular diseases, spinal cord injury, bone and cartilage repair, and autoimmune diseases.

Treatment for multiple sclerosis: A vast amount research has been conducted in recent years for the use of mesenchymal stem cells to treat multiple sclerosis. This form of treatment for the disease has been tested in many studies of experimental allergic encephalomyelitis, the animal model of multiple sclerosis, and several published and on-going phase I and phase II human trials.

Treatment requirements: Current treatments are unable to prevent the accumulation of irreversible damage to the central nervous system. Patients with multiple sclerosis experience two major forms of damage, one from on-going autoimmune induced processes and the other to natural repair mechanisms. Therefore, an ideal treatment must possess both immunomodulating properties to control irregular autoimmune responses and regenerative properties to stimulate natural repair mechanisms that can replace damaged cells.

Therapeutic mechanisms: The exact therapeutic mechanisms of mesenchymal stem cells in the treatment of multiple sclerosis are still very much up to debate among stem cell researchers. Some of the suggested mechanisms are immunomodulation, neuroprotection, and neuroregeneration.

Immunomodulation: Mesenchymal stem cells can induce the release of bioactive agents such as cytokines that can inhibit autoimmune responses. In patients with multiple sclerosis, autoreactive lymphocytes such as T and B cells cause damage to the central nervous system by attacking myelin proteins. Myelin proteins make up the myelin sheath that functions in protecting nerve axons, maintaining structural integrity, and enabling the efficient transmission of nerve

impulses. By suppressing the unregulated proliferation of T and B cells, mesenchymal stem cells can potentially minimize and control on-going damage to the central nervous system. Mesenchymal stem cells can also stimulate the maturation of antigen presenting cells. Antigen presenting cells trigger the immune system to produce antibodies that can destroy potentially harmful agents. This property allows mesenchymal stem cells to actively contribute to neutralizing harmful autoreactive T and B cells.

Neuroprotection: Mesenchymal stem cells can promote neuroprotection in the central nervous systems which may prevent the progression of chronic disability. The mechanisms include inhibiting apoptosis of healthy cells and preventing gliosis, the formation of a glial scar. They can also stimulate local progenitor cells to produce replacement cells for rebuilding the myelin sheath.

Neuroregeneration: The regenerative abilities of the central nervous system are greatly decreased in adults, impairing its ability to regenerate axons following injury. In addition to this natural limitation, patients with multiple sclerosis exhibit an even greater decrease in neuroregeneration along with enhanced neurodegeneration. They experience a significant decrease in the number of neural stem cells which produce progenitor cells necessary for normal maintenance and function. Decreases in the neural stem cells results in severe damage to the ability of the central nervous system to repair itself. This process results in the amplified neurodegeneration exhibited in patients with multiple sclerosis.

Mesenchymal stem cells have the ability to stimulate neuroregeneration by differentiating into neural stem cells in response to inflammation. The neural stem cells can then promote the repair of damaged axons and create replacement cells for the damaged tissue. Regeneration and repair of damaged axons has been shown to occur naturally and spontaneously in the central nervous system. This shows that it is an environment capable of unassisted, natural healing. Mesenchymal stem cells contribute to this regenerative environment by releasing bioactive agents that inhibit apoptosis and thus create an ideal regenerative environment.

Cardiovascular Diseases: Mesenchymal stem cells are able to alleviate heart fiber injury and prevent cardiac muscle cell death in mouse models of myocardial infarction, or heart attack, and prevent its further development. They can migrate to areas of inflammation and decrease infarction and improve cardiac function.^[8]

Brain Disorders: Mesenchymal stem cells have the potential to treat brain strokes as well. They can secrete factors that stimulate the function of brain cells, leading to neuron formation, blood vessel formation, and improved synaptic plasticity. They can also differentiate into neurons and neural cells to replace damaged cells. Behavioral tests performed in mouse models demonstrated a return back to normal brain function after treatment with mesenchymal stem cells. According to international statistics, chronic liver disease ranks #4 around the world as the top causes of mortality. Once a human liver incurs severe damage, it usually cannot repair itself naturally, thus may posing life-threatening risks to the patient. Given this, the only possible treatment options for chronic liver disease was with a dangerous complete liver transplant. Unfortunately, there are a lot of risks to consider with liver transplant surgery. The risk is mainly attributed to the tendency of rejection of transplanted organs. Sometimes the body detects the transplanted organ as a “foreign object,” or something that has not come from its own body, therefore, should be destroyed/rejected.

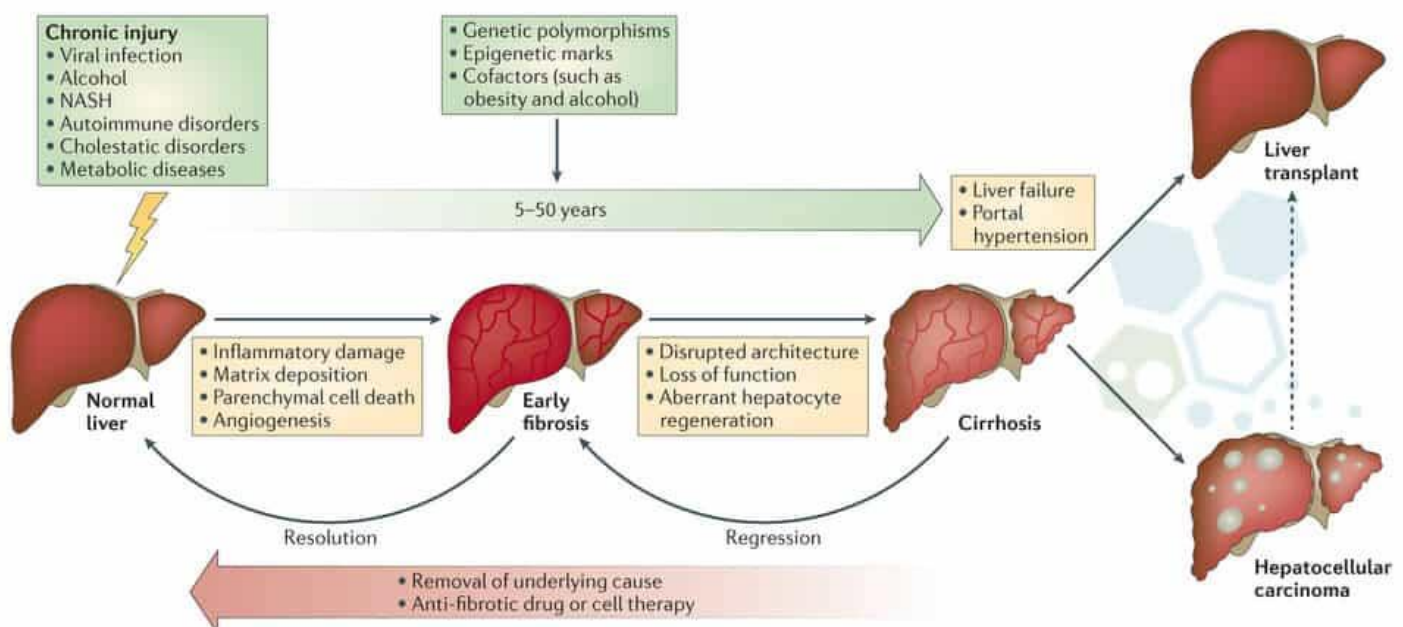


Figure-5: Different stages of liver diseases

Types of Hepatic Diseases: The liver is a complex and vital organ, and there are over a hundred various classifications of liver disease. These most common types of liver disease include:

- **Alagille Syndrome** is a genetic disorder coolly seen in young children and infants.
- **Alcohol-Related Liver Diseases**, also known as “drug-induced” or “toxic” liver failure, this disorder is typically due to overconsumption of alcohol or drugs or due to an immune reaction to certain medications.
- **Alpha-1 Antitrypsin Deficiency** is a genetic disorder that can lead to liver disease or emphysema lung failure. Patients with Alpha-1 Antitrypsin Deficiency are often misdiagnosed with Asthma
- **Autoimmune Hepatitis (AIH)** is a chronic condition that occurs when patients own immune system attacks the liver causing it to be inflamed continuously and unable to heal itself naturally. If left untreated autoimmune hepatitis can lead to liver cirrhosis and Hepatic failure.
- **Benign Liver Tumors** – Some tumors are cancerous (malignant), while others are benign (noncancerous). Noncancerous (benign) liver tumors do not spread in the body, and typically do not pose any severe health threat.
- **Biliary Atresia** is typically a genetic disorder in newborn children affecting the production of bile and the bile ducts. Biliary atresia disease caused the bile ducts to become blocked and inflamed, causing the bile liquid to remain in the liver and leading to scarring (cirrhosis) the liver.
- **Budd-Chiari syndrome** is a rare condition caused by blockage (occlusion) of the hepatic veins. Symptoms of Budd-Chiari syndrome include ascites, abdominal pain, and liver enlargement.
- **Cirrhosis of the liver** occurs with healthy liver tissue gets replaced with non-living scar tissue. This condition is usually a symptom of several other liver diseases.
- **Crigler-Najjar Syndrome (CNS)** occurs when there is high-level bilirubin (toxin) in the blood (hyperbilirubinemia). Bilirubin is the by-product of breaking down red blood cells but must be removed from the body through the bile and intestines. High levels of bilirubin in the blood can lead to jaundice and can also travel to the brain and cause brain damage.
- **Fascioliasis (Hepatic fascioliasis)** is caused by parasitic infection of the liver by a liver fluke (*Fasciola hepatica*)
- **Fatty liver disease or hepatic steatosis** occurs when triglyceride fat rapidly accumulates in liver cells.
- **Non-alcoholic fatty liver disease (NAFLD)** is a family of conditions that are usually associated with metabolic syndromes like diabetes 2 or obesity.
- **Galactosemia** is a genetic disorder that prevents patients from processing sugar galactose. Rapid accumulation of galactose in the body can result in dangerous complications such as renal failure, PKD, enlarged liver, brain damage, or cataracts in the eyes.

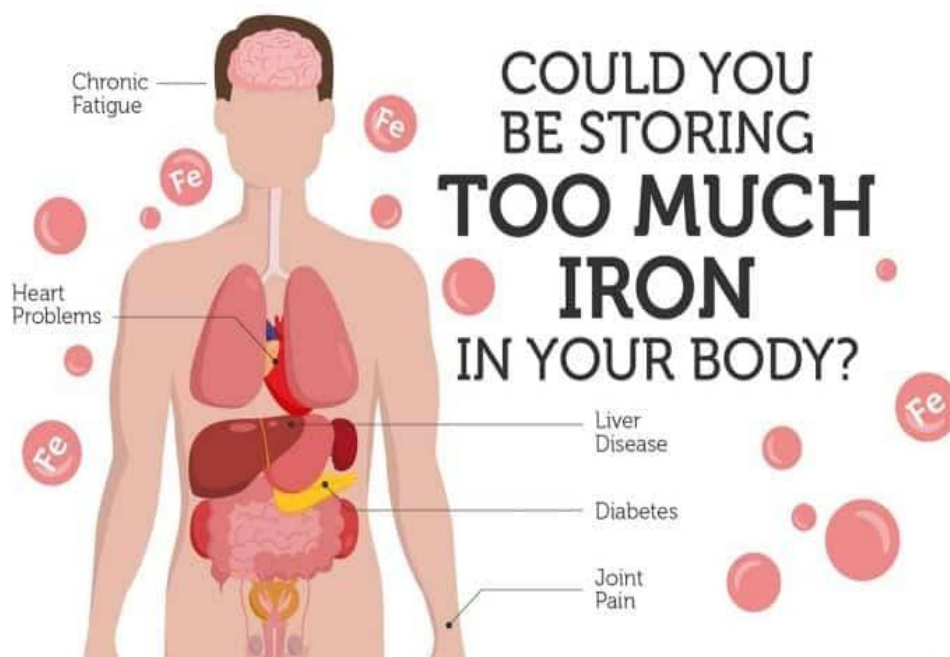


Figure-6: Excessive iron causes danger

- **Gilbert Syndrome** which is a genetic disorder that prevents the liver from processing bilirubin. This condition is more common in men than in women and can cause jaundice.

- **Hemochromatosis** is a common genetic disorder which causes patients to absorb and store too much iron in the body. The abundance of iron buildup can be very dangerous for several organs, especially the liver and if left untreated can lead to organ failure.
- **Hepatic Encephalopathy**, also known as portosystemic encephalopathy (PSE), is a medical condition that affects the brain function of patients with severe liver disease and the inability to remove toxins from the blood. If these toxins build up, they travel throughout the body until they reach the brain resulting in physical and mental dysfunction.
- **Hepatitis inflammation of the liver** can be caused by several factors, including viruses (viral hepatitis), liver toxins, autoimmune response, or hereditary conditions.
- **Hepatitis A liver disease** is caused by the hepatitis A virus (HAV) and causes the liver to get swollen and prevent it from functioning correctly.
- **Hepatitis B liver disease** is usually preventable and caused by the hepatitis B virus (HBV). A Hepatitis B diagnosis can cause the liver to enlarge and prevents proper function. Hep B is treatable, and over 95% of adults who are exposed to Hep B recover in 1 year or less. Chronic Hepatitis B is severe and can result in cirrhosis of the liver, liver failure, or liver cancer.^[9]
- **Hepatitis C liver disease** is caused by the Hepatitis C virus (HCV). Hep C can be autoimmune and causes severe inflammation in the liver, which leads to scarring and liver fibrosis. The cellular death of liver cells triggers the body to release inflammatory cells in the liver which then leads to hepatomegaly (enlargement of the liver) and also causes the Glisson's capsule (fibroelastic sheath) around the organ to stretch causing pain.
- **Hepatorenal Syndrome (HRS)** is a dangerous condition that reduces kidney function in patients with advanced liver disease. Hepatorenal Syndrome common in patients with advanced scarring in the liver and ascites but can also happen in patients with acute liver failure, fulminant hepatic failure, or other types of liver diseases.
- **Intrahepatic Cholestasis during Pregnancy (ICP)** is a condition that affects the flow of bile acids in the liver resulting in the pregnant woman to continually itch. This complication usually appears in the third trimester, when hormone concentrations are highest.
- **Lysosomal Acid Lipase Deficiency (LAL-D)** a progressive genetic disorder that restricts a patient's ability to produce lysosomal acid lipase enzyme. The body needs this vital enzyme to breakdown lipids (fats) and cholesterol. When the lysosomal acid lipase enzyme is deficient or missing, fats begin to accumulate in tissue and organs throughout the body resulting in liver disease along with high levels of LDL bad cholesterol that is linked to cardiovascular diseases.
- **Liver cysts** are benign but abnormal growths sacs in the liver that are filled with fluid
- **Liver Cancer** occurs when malignant cells in the liver grow and spread. Any cancer that starts in the Liver is called liver cancer, and Hepatocellular carcinoma (HCC) and cholangiocarcinoma are the most common type of liver cancer, and if the disease spreads from the liver to other organs, it's diagnosed as metastatic liver cancer. When pancreas cancer spreads, it typically affects the liver. Different rare types of liver cancer include hemangiosarcoma and angiosarcoma of the liver.
- **Primary biliary cirrhosis or Primary biliary cholangitis (PBC)** is a chronic autoimmune liver disease that causes damage to the intrahepatic bile ducts.
- **Primary Sclerosing Cholangitis (PSC)** liver disease is a chronic disease that slowly damages the bile ducts and then travels to the small intestine. Patients with PSC suffer from constant inflammation and fibrosis (scarring) of tissue that causes bile accumulation in the liver until it loses all ability to function correctly. PSC advances slowly, and liver failure can occur 8-15 years after initial diagnosis. Most patients with people with Primary Sclerosing Cholangitis will need a liver transplant, but stem cell therapy for Primary Sclerosing Cholangitis can be useful if applied soon after diagnosis. PSC can also result in bile duct cancer.
- **Reye Syndrome** can affect all organs in the body but especially harmful to the liver and brain. Reye Syndrome often occurs in children recovering from viral infections, like chicken pox and the flu. Complications of Reye syndrome in the Oliver can include abnormal liver function tests, fatty liver deposits, poor blood clotting, or total liver failure.
- **Wilson Disease** is a genetic disorder that causes the patient to retain excess copper deposits in the body and not release copper into bile fluid as it usually should. Copper builds up in the blood and liver can lead to damage in the kidneys, eyes, and brain. If left untreated, Wilson's disease can result in severe brain damage, Spondyloarthropathy induced hepatic steatosis, kidney failure, liver failure, and death.

Diagnosis & Genetic Testing for Liver Diseases: Blood Tests and radiology scans used to diagnose liver failure include: Liver Panels & Liver Function Test (LFT), Complete Blood Count Comprehensive Metabolic Panel (CMP), Alpha-1 Antitrypsin, Bilirubin, DCP, AFP Tumor Markers, Albumin, Total Protein, GGT, PT, AMA, AST, ALP, ALT, Copper

Test (hepatic copper concentration), Ethanol tests, Overdose Drug Testing, Iron Tests, Hepatitis A Test, Hepatitis B and Hepatitis C Tests. The regeneration center and its functional medicine department offer a comprehensive list of DNA testing options to test for inheritable liver & glycogen storage diseases, including the following panels: ATP7B, SPG70, JAG1, NOTCH2, ILLD, METRS, MRS, FBP1, SLC5A1, SLC2A2, PHKA2 G6PC, GBE1,SLC37A4, PYGL GYS2, PHKB, PHKG2,FBP, FBPase 1 & Rpl17. To help reduce the risk of developing liver disease, consider making changes to your diet, and get regular exercise.

Early Symptoms of Liver Disease

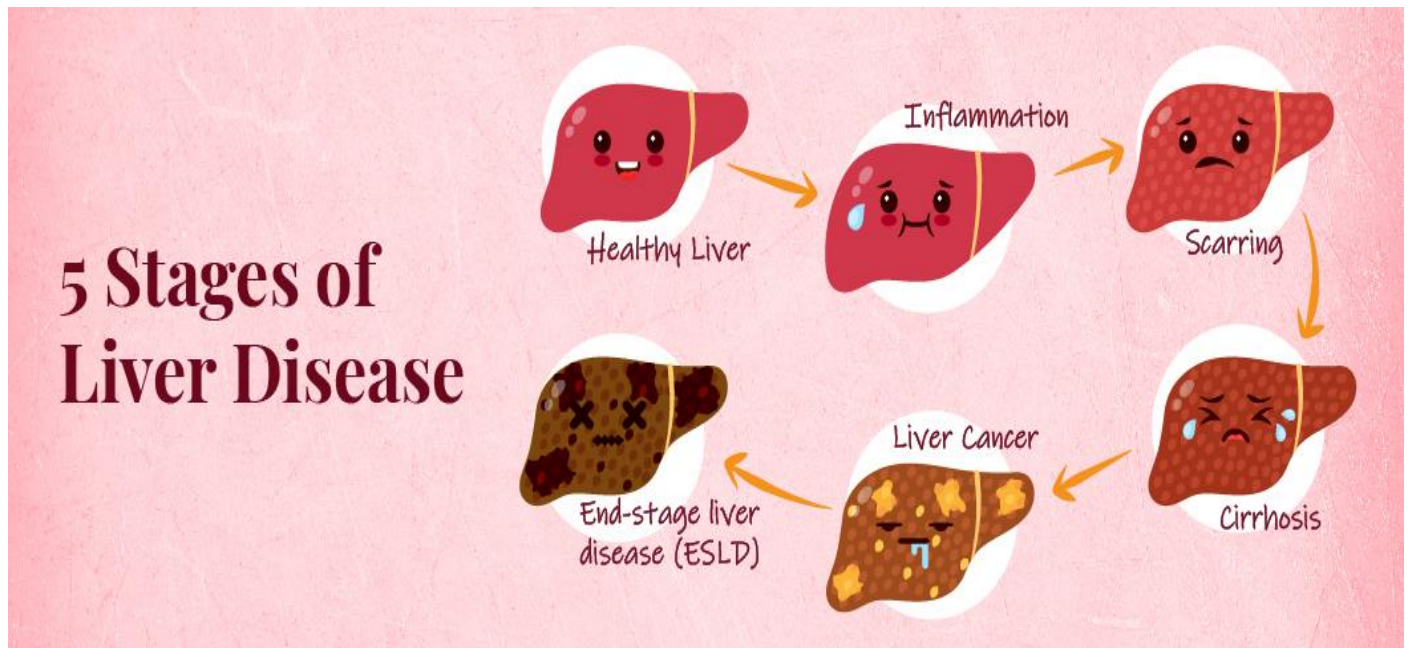


Figure-7: Stages of Liver Diseases

Common Causes of Liver Disease: It's widely accepted that most of the common causes of chronic liver disease are modifiable before they become an issue by altering you're eating and health habits. The best treatment plans always start with prevention. Healthy living practices will be crucial after treatment to avoid relapse and chronic liver disease. The Liver is the largest organ in the human body and is fully capable of regenerating itself under normal conditions. Since the Liver has many responsibilities such as detoxification of the blood, fighting infections, and aiding in the digestion of foods, it can severely impact the overall health of a person if it becomes diseased.

Organ donors are very limited, and the prices for liver transplants is very high, and the surgery itself involves many risks. Thankfully, our internationally trained stem cell regeneration doctors were able to create a more modern approach to healing. Adult stem cell therapies help induce the rapid production of hepatocytes in a patient with chronic liver disease or Cirrhosis of the Liver. ^[10]

Liver Disease Treatment with Stem Cells

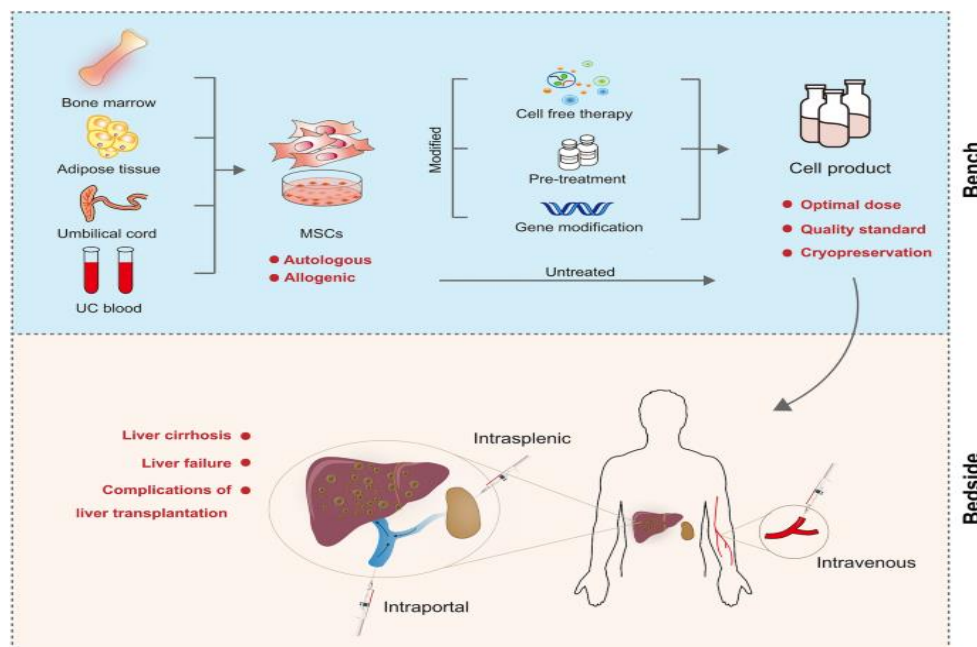


Figure-8: Liver treatment with stem cells

Enhanced mesenchymal stem cells (MSC+) offer an attractive therapeutic solution to reverse *hepatic* cirrhosis and liver diseases based on many factors, including:

- Immunomodulatory properties
- Ability to differentiate into hepatocytes, biliary epithelial cells and liver progenitor cells
- Natural replacement of damaged hepatocyte cells
- Reduction of pro-inflammatory cytokines
- Reversing hepatocellular damage and repair hepatic ducts & hepatic plates
- Promote persistent hepatocyte regeneration
- Capacity to inhibit cell activation
- Stop Fatigue. Jaundice. Constant Itching.
- Increases rate of apoptosis (a paracrine mechanism)

Conclusion: With recent advancements in regenerative medicine for failed kidney, heart attacks, and diabetic nephropathy, there is finally an effective alternative liver regeneration treatment to repair fatty liver & chronic liver disease using targeted protocol of liver cells and growth factors. *Liver stem cell therapy* can help reduce inflammation and liver fibrosis through the paracrine regeneration and immunomodulation functions on liver cells & CD4+ T cells. These combined effects are expected to play a favorable role in the restoration of liver homeostasis. Chronic Parkinsonism is also known to be associated with liver cirrhosis. The liver is generally made up of cells called hepatocytes. The enhanced liver cells are capable of replicating so that new hepatocytes will be replacing old hepatocytes naturally after 200 to 300 days of use. If a patient's liver is severely damaged, the natural replicating process cannot be completed by the hepatocytes. This is where oval cells come to the rescue. It is believed that oval cells are also capable of producing new hepatocytes should the need arise. Combo treatment for Liver failure is done as an outpatient procedure and looks to introduce therapeutic levels of hepatocytes liver cells or Hepatic oval cells use the paracrine mechanism to initiate in the healing of damaged tissues and cells forcefully. Combination MSC+ Hepatic Cell treatment protocol can also be done by using expanded Adult multipotent stem cells and hepatic growth factors. Multipotent stem cells are capable of differentiating into different kinds of cells, including hepatocytes (liver cells).

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