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### **Adipose-derived stem cells in the treatment of hepatobiliary diseases and sepsis**

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#### **Abstract**

Determination of the mesenchymal stem cells is one of the greatest and most exciting achievements that tissue engineering and regenerative medicine have achieved. Adipose-derived stem cells are easily isolated and cultured for a long time before losing their stem cell characteristics which are self-renewal and pluripotency. Adipose-derived stem cells (AD-MSC) are mesenchymal stem cells that have pluripotent lineage characteristics. They are easily accessible and the fraction of stem cells in the adipose tissue lysates is highest among all other sources of mesenchymal stem cells. It is also HLA-DR negative and can be transplanted allogeneically without the need for immunosuppression. These advantages have popularized its use in many fields including plastic reconstructive surgery. However, in the field of hepatology and liver transplantation, the progress is slower. Adipose-derived stem cells have the potential to modulate inflammation, ameliorate ischemia-reperfusion injury, and support liver and biliary tract regeneration. All these are very important for the treatment of various hepatobiliary diseases. Furthermore, the anti-inflammatory potential of these cells has paramount importance in the treatment of sepsis. We need alternative therapeutic approaches to treat end-stage liver failure. AD-MSC can provide a means of therapy to bridge to definitive therapeutic alternatives such as liver transplantation. here we propose to review theoretic applications of AD-MSC in the treatment of hepatobiliary diseases and sepsis.

## INTRODUCTION

Stem cell therapy provides limitless therapeutic options in the field of medicine which is the direct result of the achievements obtained by the field of regenerative medicine. The therapeutic applications are early in its stages and the clinical trials are ongoing [1]. Ideal stem cell should have ability of self-renewal, multilineage capacity, should be easily isolated and cultivation conditions should be simple [2,3]. Mesenchymal stem cells (MSC) have been used abundantly for this purpose [3,4]. Mesenchymal stem cells have an intricate cell biology and are amenable to being utilized in tissue engineering. They secrete various potent growth factors and cytokines, they have pluripotent differentiation capabilities and they are abundant in the body including the bone marrow, oral cavity and adipose tissue [5].

In 2001, Zuk and colleagues [6] have isolated and defined the **stromal vascular fraction (SVF) of adipose tissue**. SVF contains **a mixture of erythrocytes, fibrocytes, endothelial cells, smooth muscle cells, pericytes of vascular origin, and fat cells**. SVF can be cultured, forming a fibroblast like cells that are adherent to the culture flask. These cells were originally named as the pre-adipocytes [7,8]. However, it has been shown that these cells have mesodermal multipotent differentiation ability and they are currently called the Adipose Derived Mesenchymal Stem Cells (AD-MSC) [7]. Currently, advancements in tissue engineering and regenerative medicine have shown that these cells can differentiate into cells and tissues of endodermal, mesodermal and ectodermal origin [1,2]. There are some advantages to using the AD-MSC in regenerative medicine. The most important one is the abundance of stem cells in the adipose tissue [9,10]. Adipose tissue contains at least 100 times higher amount of stem cells when compared to other sources such the bone marrow [1,11]. Furthermore, the isolation procedure is very simple and efficient [1,2,6,7,12]. Obtaining the fat tissue from individuals are very easy. The proliferative capacity and durability of AD-MSC excel the MSC obtained from other sources [2,13-15].

Isolation procedure is very simple and has been done for a long time <sup>[16]</sup>. It includes mechanical disruption of the tissue followed by enzymatic digestion and ultracentrifugation (*Figure 1*). It is cultured in standard cell culture media without the need of a special culture media. During the standard culture of eukaryotic cells 10% (v/v) fetal bovine serum (FBS) is used. This can lead to certain problems such as immune reactivity and transmission of zoonotic infections<sup>[17]</sup>. For this reason, platelet rich plasma (PRP) can be used as an alternative to FBS. PRP has also been shown to enhance AD-MSC growth *in vitro* <sup>[18]</sup>.

There is a uniform pattern of surface marker expression for AD-MSC which are presence of CD90, CD73, CD105, and CD44<sup>[19,20]</sup>. Expression of CD34 and CD49d is highly reserved for AD-MSC and is absent in other MSC types <sup>[21]</sup>. The secretion profile of AD-MSC includes a wide range cytokines, chemokines, and growth factors. The effects of the secreted factors are paracrine in their activity. There are factors that promote angiogenesis such as fibroblast growth factor 2 (FGF-2), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and insulin-like growth factor 1 (IGF-1) <sup>[22]</sup>. Also, Matrix metalloproteinase- (MMP-) 3 and MMP-9 contribute to their proangiogenic activity <sup>[23]</sup>. Their effect on the system is usually induction of immunoregulatory type changes promoting tissue injury and angiogenesis. The factors that are responsible for the immune effects of AD-MSC are macrophage-colony stimulating factor (M-CSF), granulocyte-colony stimulating factor (G-CSF), interleukin- (IL-) 6, tumor necrosis factor (TNF), and prostaglandin E2 <sup>[23]</sup>. Therefore, there is T helper type 2 (Th2) polarization of CD 4 positive T-cells and M2 polarization of the macrophages. All these changes reduce inflammation and increase the wound healing capacity of the tissues <sup>[24,25]</sup>.

In acute and chronic liver failure. the regenerative capacity of the liver is overwhelmed by the noxious stimuli <sup>[26,27]</sup>. Therefore, regeneration or repair of the liver is very complicated due to the presence of variety of parenchymal cells <sup>[28]</sup>. These cells include the hepatocytes, cholangiocytes, hepatic stellate cells and immune cells including the Kupffer cells, Natural Killer, Natural Killer T cells, eosinophils <sup>[28,29]</sup>.

In summary, through the paracrine effects of the AD-MSC derived cytokines, chemokines, and growth factors, AD-MSC stimulate angiogenesis, exert antiapoptotic effects, recruit other MSC and progenitor cells to the site of injury<sup>[27,30,31]</sup>. In addition, they stimulate the proliferation and differentiation of the wide range of cells present in the site of injury. They also reduce the reactive oxygen species (ROS) in the microenvironment and reduce ROS mediated injury to the tissues<sup>[27,30,31]</sup>. One unique feature of MSC is their ability to fuse with parenchymal cells in the injury site to promote intercellular interaction and exchange cellular macromolecules through the intercellular nano-channels that are formed <sup>[27,30–32]</sup>.

The aim of the present study is to summarize the current literature in terms of AD-MSC in the cellular therapy for hepatic, biliary regeneration. Also, we will try to briefly summarize the role of AD-MSC in the treatment of septic conditions. We hope that all these will help the readers to grasp the potential of AD-MSC in treatment of hepatobiliary diseases.

### **AD-MSC IN HEPATIC DISEASE AND REGENERATION**

The immune regulatory and anti-apoptotic effects of AD-MSC aid regeneration of the liver and help healing of the liver injury caused by viral infections, toxins, and genetic diseases <sup>[33,34]</sup>. Studies have shown that AD-MSC express liver specific markers even if they are not targeted *in vitro* <sup>[35]</sup>. There are many sources for AD-MSC, and each have different biological behavior. Liver falciform ligament derived AD-MSC show higher proliferative capacity, higher embryonic stem cell capabilities <sup>[36]</sup>. Falciform ligament is readily available during liver surgery and can be used to enhance healing of the tissue following liver surgery. Surgeons have been using falciform ligament flaps to support anastomosis or to fill a gap in liver following resection for a long time <sup>[37]</sup>. This may be attributed to the enhanced healing capacity of the stem cells present in the falciform ligament.

Experimental studies are abundant showing reduced inflammation, support of hepatic regeneration and normalization of metabolic derangements in liver failure experimental

models. We will try to briefly summarize some of the cornerstone experiments that are present in the literature. Experimental studies have shown that condition of the host determined the type of differentiation of the AD-MSC. In experimental model of acute liver failure, it has been shown that AD-MSC showed increased expression of specific markers for hepatocytes [38]. AD-MSC have been shown to be amenable to *in vitro* targeting to hepatocytes which can later be used to treat an experimental model of acute liver failure [39]. Transplantation of AD-MSC 24 h before 70% hepatectomy model in rats, ameliorated hepatic dysfunction and improved liver regeneration by normalizing the metabolic processes in the liver [40]. Banas *et al* [41] has also reported their results in a carbon tetrachloride treated acute liver failure model. They have shown that treatment with AD-MSC that were preconditioned *in vitro* ameliorated the liver failure and normalized liver function tests in animals in the treatment arm [41]. AD-MSC give as treatment after the development of acute liver failure has also been shown to be effective in improving liver regeneration and functions [42]. Preconditioning of AD-MSC has resulted in development of functional liver tissue (liver bud) in experimental models [43]. AD-MSC were shown to significantly inhibit the proliferation and activation of and also, promote the programmed cell death of HSCs there by reducing hepatic fibrosis in experimental models [44,45].

AD-MSC can also be used together with nanoparticle technology to increase the engraftment rates and enhance the efficacy of the AD-MSC in reversal of liver injury and liver fibrosis [43]. Furthermore, use of liver bioscaffolds have been shown to support the growth of neonatal multilineage progenitor cells in to fully functional liver tissue [46]. If stem cells are used during the recellularization process, the results seem to be better when compared to primary parenchymal cells such as the hepatocytes [43]. Apart from supporting the regenerative process, AD-MSC reduce the ischemia and reperfusion injury during liver surgery and diseases. It has been shown that AD-MSC reduce ischemia reperfusion injury in liver by reduction of various inflammatory cytokines such as IL-1 $\beta$ , IL-6, and TNF [47]. Furthermore, AD-MSC secrete counter regulatory cytokines such as IL-10 and also, secrete factors such as hepatocyte growth

factor (HGF) and cyclin D1 which are effective in hepatic regeneration <sup>[47]</sup>. The effects of AD-MSC include immune regulation, reduction of oxidative and inflammatory tissue destruction and regeneration of the parenchymal cells. The proposed mechanisms of action of AD-MSC are summarized in *Figure 2*. The clinical trials so far has been successful and have shown a good safety profile of AD-MSC in humans. It prevented acute-on-chronic liver failure and improved liver functions in patients with cirrhosis with various etiologies <sup>[48-50]</sup>. The summary of the preclinical studies and clinical trials are summarized in *Table 1* and *2*.

### **THE ROLE OF AD-MSC IN BILIARY REGENERATION**

Liver resection and transplantation are among the definitive treatments of life-threatening chronic liver disease and primary/secondary liver tumors. The most frequent complication following liver disease is the biliary complications <sup>[51,52]</sup>. Some of these complications may even cause mortality in the patients. Stenosis is one of the biliary complications that are observed following hepatobiliary surgery. In major surgeries like living donor liver transplantation, it has been reported to affect 10-30% of the patients <sup>[53]</sup>. Treatment of this complication requires repeated procedures, restorative operations, and frequent prolonged hospitalizations. Majority of the complications are due to ischemia and reperfusion injury. Ischemia reperfusion injury has adverse effects on both hepatic and biliary regeneration <sup>[54,55]</sup>. Zhu *et al* <sup>[55]</sup> have reported that warm ischemia times exceeding 20 minutes were associated with biliary complication and biliary epithelial damage in an experimental model. The cholangiocytes have pluripotent differentiation potential, but it is overwhelmed during ischemia and reperfusion injury <sup>[54,55]</sup>. MSC therapy may be an alternative or adjunct to conventional therapies for biliary complications. AD-MSC are preferred alternatives for they are easily accessible, and they promote anti-inflammatory mechanisms and regeneration in the tissue which may be beneficial for biliary regeneration <sup>[55,56]</sup>. There are limited studies regarding the versatility of AD-MSC in biliary regeneration <sup>[47,57]</sup>. Hara *et al* <sup>[57]</sup> have shown that AD-MSC sheets were effective in preventing biliary

strictures in duct-to-duct anastomoses. However, the studies are limited regarding the role of AD-MSC in biliary regeneration. Further studies will provide innovative therapeutic options for biliary complications.

### **THE ROLE OF AD-MSC IN THE TREATMENT OF SEPSIS**

Sepsis is an overwhelming inflammatory response to invading microorganisms. The severity of the disease depends on the virulence of the microbial pathogen, amount of toxins secreted by the pathogen and the physiologic status of the host. The recovery from sepsis depends on the balance between the pro-inflammatory cytokines and anti-inflammatory mechanisms that counterbalance inflammation [58]. TNF- $\alpha$  and IL-6 are the potent pro-inflammatory cytokines that have major role during the pathogenesis of sepsis [59,60]. As our understanding of the physiopathology increases, alternative immunomodulatory therapies are being developed and investigated for clinical use [61]. Currently, MSC are being used for the treatment of sepsis. Majority of these are isolated from the bone marrow which is not an easy process [62]. AD-MSC have been shown to be effective in endotoxemia induced sepsis models in rats by reducing apoptosis and the rate of multi-organ failure [63]. The anti-inflammatory action of MSC can be a direct effect through cell-to-cell interaction or may be through paracrine effects of the secreted mediators or secretion of exosomes/microvesicles to the inflammatory microenvironment [62]. MSC have been shown to reduce pro-inflammatory cytokines and increase cytokines such as IL-10 and induce a regulatory phenotype in the immune cells [64]. Through this mechanism, MSC reduce the amount of macrophage and neutrophil infiltration in target organs such as the lungs, kidneys and the liver; thus reducing the risk of multiple organ failure [62,65–67]. MSC also increase the phagocytic activity of monocytes in circulation and reduce the effective microbial concentrations [68].

In sepsis or viral pneumonia such as the one seen in corona virus related infectious disease-2019 (COVID-19), traditional therapeutic options were insufficient [69]. Bone-marrow derived MSC played an important role for both reduction of inflammatory



damage in end-organs and also in clearance of microbial agents from the circulation of the patients. The studies regarding the role of AD-MSC in sepsis are not enough and further studies are needed. The proposed mechanism of action of AD-MSC in hepatobiliary diseases and sepsis are summarized in *Figure 2*.

## **CONCLUSION**

There is a lot of unknown points regarding the role of AD-MSC in the treatment of hepatobiliary diseases. However, the results of preclinical studies and limited clinical trials are promising. It seems to be a good alternative treatment to bridge acute or acute-on-chronic liver failure until a definitive liver transplantation can be performed. Furthermore, it may promote wound-healing process preventing many complications in biliary tract following major liver surgeries.

The utility of AD-MSC in the treatment of hepatobiliary disease and sepsis is relatively new. Brief reports are showing the efficacy of AD-MSC in controlling inflammation and regenerating parenchymal tissue. However, there is no established firm protocol. The dose and dosing intervals of the allogenic AD-MSC transplantation requires further research for establishing universal protocols. Furthermore, the role of targeted or genetically modified AD-MSC are unknown. Bioscaffolds may also provide modeling of the tissue and providing precursors for the liver and biliary tract. Combination of AD-MSC with nanoparticles for potentiating the anti-inflammatory response will be an important area of research in the future. Therefore, further research is needed to guide the physicians for future innovative clinical applications.

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