Chapter 12

Regenerative Medicine in Liver Cirrhosis: Promises and Pitfalls

Asima Tayyeb, Fareeha Azam, Rabia Nisar, Rabia Nawaz, Uzma Qaisar and Gibran Ali

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Abstract

Liver cirrhosis is irreversible and mostly ends up with complete loss of liver function/end-stage liver failure, and the only proven treatment is liver transplantation. Scarcity of donor, high cost, lifelong immunosuppression, and surgical complications are the major issues associated with liver transplantation and these urge to look for alternate therapeutic approaches. Advancements in the field of regenerative medicine are arising hope for the treatment of liver cirrhosis. This chapter deals with the scope of liver regenerative medicine in the treatment of liver cirrhosis. Review of the literature showed that liver regenerative medicine no doubt holds great promises and added a lot of hope to the cure of liver diseases. Primarily, cell-based therapies had shown great potential to treat liver cirrhosis. Successful clinical human trials further strengthen their significance in the field. However, recent trends in liver regenerative medicine are focusing on the development of tissue engineering leading to generation of the whole organ. Despite advantages, liver regenerative medicine has several limitations and sometimes been over-optimistically interpreted. In conclusion, the current scenario advocates to conduct more preclinical and clinical trials to effectively replace liver transplantation with liver regenerative medicine to treat liver diseases.

Keywords: regenerative medicine, stem cells, hepatocytes, tissue engineering

1. Introduction

Liver is one of the largest and most important metabolic organs in the human body with considerable regeneration capacity. However, in prolonged hepatic injuries, the regeneration capacity of hepatocytes times out and a cascade of life-threatening complications is initiated

leading to liver cirrhosis. Liver cirrhosis is irreversible and mostly ends up with complete loss of liver function/end-stage liver failure. End-stage liver failure with high rates of morbidity and mortality poses a significant threat to human health as well as economy throughout the world [1]. As current pharmacological treatments are inefficient to reverse this loss, liver transplantation is the only effective lifesaving option. Since the first liver transplantation in 1963, the number of cases requiring transplantation are considerably increasing with the passage of time. Despite the success of liver transplantation, there is a gap between demand and supply. Only 30–50% of annual liver donation desires are fulfilled and at least about 15% patients die while being on the waiting list [2, 3]. Besides scarcity of liver donors, high cost, postoperative graft rejection, and long-term immune-suppression are few more serious constraints associated with liver transplant [4]. Therefore, it is crucial to look for effective and operative alternate approaches of liver transplantation.

Advancements in the field of regenerative medicine open up new horizons and arising hope in the treatment of irreversibly damaged liver cirrhosis. Liver regenerative medicine mainly emphasizes on the establishment of new therapies to either functionally restore the chronically damaged liver tissue or to develop the entire new organ [5]. Elucidation of cellular and molecular mechanisms during the last couple of decades in the field of hepatic organogenesis and regeneration provides milestones in the development of liver regenerative medicine. Moreover, compared to current operative therapies, it is less invasive, is less expensive, and avoids the problem of shortage of donors, immune rejection, and other similar complications. Ideally, liver regenerative medicine seems an ultimate solution for liver cirrhosis.

Liver regenerative medicine uses two key approaches based on cell therapy and tissue/organ engineering. Cell-based therapy is defined as the transplantation of cells from different sources with or without differentiation to improve liver function [6]. Transplantation of mature hepatocytes and liver stem/progenitor cells (LSPCs) from allogeneic sources is already in clinical trials. However, current research is intended to overcome the problem of immune rejection associated with allogeneic sources and focuses on therapies based on generation of autologous hepatocytes from MSCs and induced pluripotent stem cells (iPSCs) [5]. Elucidation of cell type, which can be successfully differentiated into functional and transplantable hepatocytes or liver progenitor cells, is another major task under study [7]. Furthermore, researchers are trying to refine protocols for proliferation, differentiation, and storage of these cells to have them in plenty and always ready to be transplanted.

Second strategy mainly covers the area of liver tissue/organ engineering, engraftment, and monitoring in patients. Ongoing therapeutic approaches in tissue engineering include implantable constructs of hepatic tissues and whole organ. For the construction of hepatic tissues, natural and synthetic bioactive scaffolds are designed [5]. Nanotechnology and microchip devices are contributing a lot in this lane. Moreover, whole organ engineering is also in great focus to escape end-stage liver diseases. However, determination of ideal cell types, cell volume, and optimal seeding techniques is yet to be discovered [8, 9].

This chapter deals with the scope of liver regenerative medicine in the treatment of liver cirrhosis. Different operative and proposed therapies along with their pros and cons are the major focus of this section and are reviewed in detail.

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2. Hepatic organogenesis

Zygote is the only totipotent structure that leads to the development of blastocyst. Blastocyst carries both embryonic and extraembryonic (inner cell mass) cell population. Inner cell mass (ICM) forms three germ layers: exoderm, mesoderm, and endoderm. Embryonic liver develops from the endodermal layer during ventral foregut closure in the midgut [10]. Cells residing in the hepatic bud are bipotent and are called hepatoblasts. Hepatoblasts are columnar in shape, release α-fetoprotein, and differentiate into mature hepatocytes and cholangiocytes [11].

Wingless type (wnt) signaling pathway, together with activin-A, plays a crucial role in the establishment of endoderm during primitive streak formation and differentiation of liver precursor cells toward hepatoblasts [12, 13]. Other key factors involved in hepatic fate determination are fibroblast growth factors (FGFs) released from cardiac mesoderm and bone morphogenetic proteins (BMPs) released by septum transversum mesenchyme [3]. Furthermore, oncostatin M and hepatocyte growth factor (HGF) control the differentiation of hepatoblasts toward hepatocytes [14], whereas Jagged-Notch signaling pathway is responsible for the development of cholangiocytes [15].

Gradually, as the liver development proceeds toward the final stages of maturation, hepatoblast number reduces markedly. Liver becomes populated with mature and unipotent hepatocytes and cholangiocytes. The remainder resident cells of liver, that is, Kupffer cells, stellate cells, and endothelium, are mesodermal in origin. Majority of the liver functions are performed by hepatocytes. On the onset of any hepatic insult, adult liver cells undergo apoptosis that calls for the replacement of lost cells or in other words liver regeneration. The schematic diagram of liver organogenesis from endodermal layer along with important molecular signaling pathways involved in activation or suppression of each step has been represented in **Figure 1**.

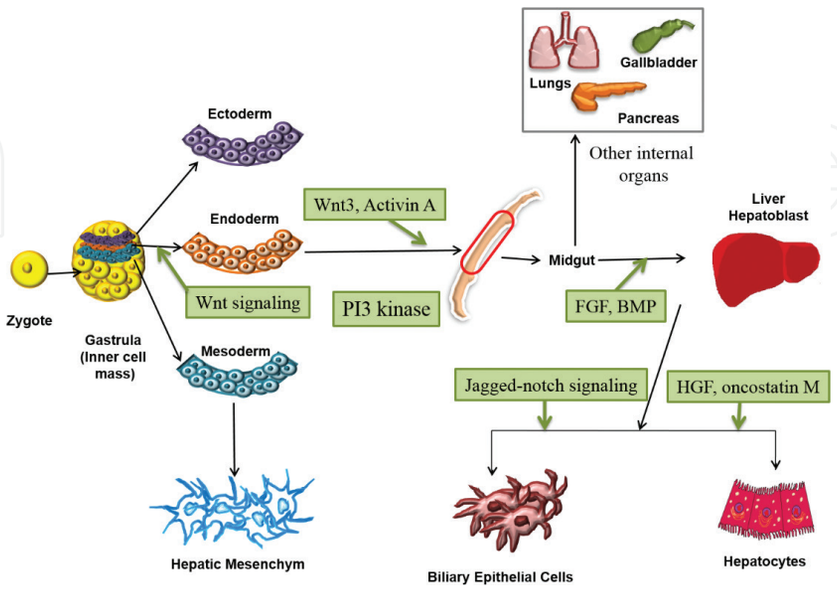

Figure 1. Schematic diagram of liver organogenesis. Molecular signals involved in the activation of each stage are indicated in the boxes occuring at various steps of liver organogenesis.

3. Liver regeneration

Elucidation of the cellular and molecular mechanisms involved in liver regeneration provides vital scientific grounds for liver regenerative medicine. Depending upon the origin of liver damage, different kinds of repair mechanisms are operative [16]. Various surgical and toxin-mediated injury models for liver regeneration have been established so far. One of the established and utterly studied model of regeneration is rodent partial hepatectomy [17]. In partial hepatectomy model, liver can regenerate to its normal size in 3–10 days even if two-thirds of its mass is surgically removed. A fine coordination of cellular and molecular events occurs in the regeneration process of partial hepatectomy. Robust hepatocyte replication followed by hypertrophy has been revealed as an underlying cellular mechanism in partial hepatectomy recovery. This vigorous change in hepatocytes is also accompanied by alteration of gene expression patterns, instigation of transcription factors, and release of growth signals. More than 100 genes are activated in an early response manner. At least 40% of these early response genes are activated by interleukin-6 (IL-6) signaling which itself is activated by tumor necrosis factor-α (TNF-α)-mediated NFκB (nuclear factor kappa-B) activation [18, 19]. The recovery of liver mass and function of living donor and recipient of liver transplantation in humans seems to adopt a similar track.

Besides utilizing mature hepatocytes for liver regeneration, another likely approach is the use of liver progenitor cells (LSPCs). They are capable of converting into different cell lines found in liver, that is, hepatocytes, oval cells, and stellate cells [20]. LSPCs got experimental and clinical support when they were overproliferated in case of induced liver injury by acetaminophen and slowly proliferated in case of liver cirrhosis [21, 22]. At present, the main focus is on the regenerative capacity of LSPCs when hepatocytes run out of their regenerative potential. LSPCs are also proved potential progenitor cells of biliary epithelium in vitro, but no specific LSPC markers are identified as yet. It seems that LSPCs are driven by the activation of certain genes and the combination of growth factors. Crucially important genes include Leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR5) and the cytokine tumor necrosis factor-like weak inducer of apoptosis (TWEAK), a member of the tumor necrosis factor (TNF) superfamily [23]. Some other mitogenic factors also play a crucial role, for example, HGF, epidermal growth factor (EGF), TGF-α, and fibroblast growth factors 1 and 2 (FGF1 and FGF2) [24]. However, there is lack of evidence pertaining to in vivo differentiation of LSPCs into hepatocytes. The articles published in 2014 used different methodologies to trace the fate of liver progenitor cells. They utterly rejected the concept of regenerative capability of LSPCs into hepatocytes. Besides, despite lack of proof of the in vivo hepatogenic differentiation of LSPCs, they surely can give rise to hepatocyte-like cells in vitro [20]. Research in this arena is ongoing and there is a probability that even in mice a part for oval cells/LSPCs in regeneration will be found.

Third major concept in liver regeneration is through extrahepatic cells that is hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) derived from bone marrow. HSC and MSC from bone marrow reach the liver via blood circulation. These HSCs and MSCs can populate the liver after hepatogenic differentiation [25]. It is proposed that these bone marrow-derived stem cells are not directly converted into hepatocytes rather they first mix

with resident liver cells and then participate in liver repopulation [26]. It has also been suggested that MSCs with multilineage differentiation potential provide a great variety of cells for nonhematopoietic tissues like liver tissues [27]. Though they are highly heterogeneous in nature, only a little fraction of it contributes to liver regeneration [28]. It is notable that bone marrow cells take part in the regeneration of liver endothelium. Twenty percent of the liver endothelial cells are made by the bone marrow-derived endothelial cells [29]. There is a need of concerning involvement of bone marrow-derived stem cells in liver parenchyma regeneration, for designing the methods for cellular therapy of liver disease [16].

4. Cell-based therapies for regeneration of liver cirrhosis

Cell-based therapies are the oldest and most efficient method to regenerate damaged liver. Effective engraftment and proliferation of donor cells in the recipient liver are the main issues of concern for liver regeneration through cell-based therapy. Depending on the donor source, cells can be of autologous [30], allogeneic, or syngeneic nature [31]. The cells are injected into the recipient through portal vein, peripheral vein [30], and intraspleenic [32] or intraperitoneal route. To enhance the transplantation efficiency, conditioning of recipient liver with partial hepatectomy [33, 34], liver irradiation [35, 36], or portal embolization [37] has been recently proposed. Broadly, cells are categorized into two main categories; stem cells and mature hepatocytes are the potential cell-based therapies adapted to date in the cure and regeneration of liver cirrhosis [5]. The roles of these cell-based therapies are shown in **Figure 2** and are discussed one by one in detail in the following section.

4.1. Hepatocytes and liver regeneration

Liver is chiefly composed of hepatocytes. Hepatocyte proliferation plays a distinctive role in liver regeneration under both acute and chronic injury conditions. The unique characteristic

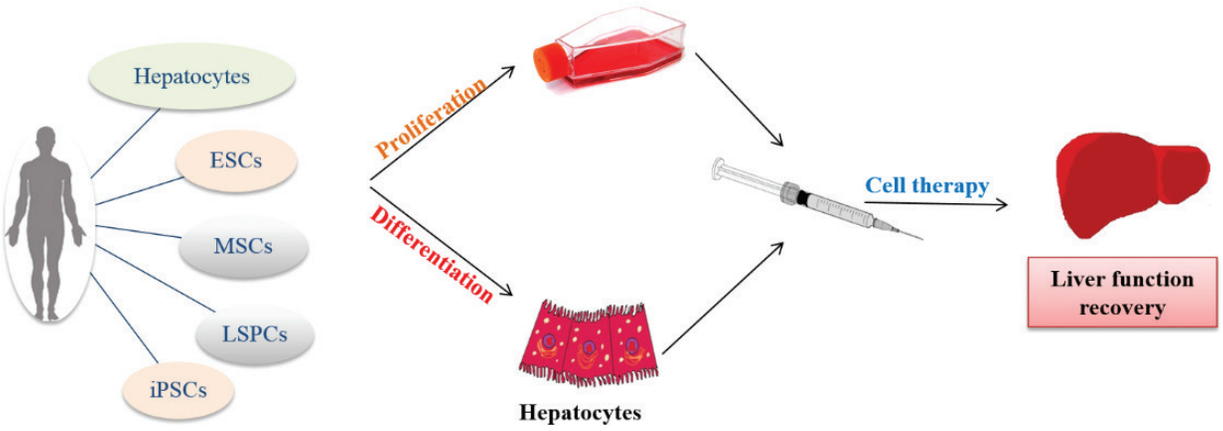

Figure 2. Different types of cells and their mode of application for cell-based therapies of liver cirrhosis. Different types of cells isolated from humans and being used in liver regeneration are shown on the left side of the figure. Each of the cell type has been injected and has recovered liver functions either through only in vitro proliferation (hepatocytes), via differentiation toward hepatocytes (ESCs and iPSCs) or through both (MSCs, LSPCs).

of hepatocytes to proliferate under stress conditions makes them ideal cell type for cell-based therapies. Primary hepatocytes were the very first type of cells to be used for cell-based therapy of liver. Isolated hepatocytes are infused either directly into the liver or into the spleen from where they can migrate to and settle down in the liver. The hepatocyte transplantation has shown to considerably improve the hepatic functions even in end-stage liver failure [38]. Typically, hepatocytes are harvested from the livers that are not suitable for transplantation [39]. However, due to problem of immune rejection, it was also tried to isolate hepatocyte from patient's biopsies [40].

Although primary hepatocytes are ideal for use in liver regeneration, this approach is prone to certain limiting factors. Inadequate supply of the required cells, slow in vitro proliferation rate [18], dedifferentiation within 72 hours of culturing [41], susceptibility to freeze-thaw damage, and loss of certain characteristic features in culture conditions are major obstacles that hinder the utilization of these cells for liver regeneration [38]. The isolated primary hepatocytes are of low quantitative value, and an autologous isolation of this cell population involves patients' inconvenience. Typically, hepatocytes are harvested from the livers that are not suitable for transplantation, so the quantitative and qualitative values of obtained cells vary considerably. All of these constraints have played a pivotal role in shifting focus toward alternate cell-based therapies.

4.2. Stem cells in liver regeneration

With the therapeutic focus being set on the establishment of personalized medicine and the replacement or regeneration of damaged tissue, stem cell-based therapies may provide a strong platform. The properties of indefinite cell division and differentiation potential into other cell types make the stem cells an ideal choice for cure and regeneration of liver cirrhosis. Another important property of stem cells is their ability to create and provide a favorable environment for growth of primary hepatocytes and/or hepatocyte-like cells [5]. Coculturing MSCs with primary hepatocytes results in their improved viability and function by providing structural and paracrine trophic support [41–43]. Moreover, stem cell therapy holds great potential especially in the cure of inherited liver diseases, where, together with gene therapy, it may correct metabolic disorders permanently without even using immunosuppressive drugs [5]. Chiefly, two approaches of stem cell-based liver regeneration are in practice either their direct injection or in vitro differentiation toward hepatocyte-like cells and transplantation.

Some types of stem cells show efficient growth in vitro, could be a rich pool to supply hepatocytes/precursor cells, and thus be used largely for transplantation. If the wide availability of human hepatocytes is made possible, this could be a major breakthrough in the treatment of various liver diseases. However, the research work debating good capacity stem cell therapy lack in reproducibility evidence or some of these even have been overoptimistically interpreted. Another important milestone is to decide on the preference of stem and precursor cell types. It is a difficult task to compare different cell types with respect to their reported capacity of differentiation toward hepatocytes [44]. We therefore discuss the possibilities these cell therapies offer one by one, along with the limitations which are making these feats harder to achieve.

4.2.1. Embryonic stem cells and hepatocyte generation

Differentiation of cultured embryonic stem cells toward hepatocyte-like cells in vitro appears to be the most studied model of mature hepatocyte generation. In mouse models of liver injury, hepatocyte-like cells not only recover the liver by proliferation but also provide trophic factors that assist the endogenous hepatic regenerative capability [45]. Human ESCs efficiently form embryoid bodies in suspension cultures forming three germ layers [46]. Hepatocyte isolation from this heterogeneous cell population is very difficult, suggesting endoderm enrichment to be a practical option with maximum hepatocyte yield.

A directional differentiation strategy for the generation of functional hepatocytes from embryonic stem cells involves sequential supplementation of various molecular factors (growth factors and cytokines necessary for development of human embryonic liver)-enriched growth medium. The molecular factors involved in early embryonic differentiation such as fibroblast growth factor (FGF2/4), bone morphogenetic protein (BMP2/4), activin A and Wnt3 can be used for endoderm enrichment from cultivated embryoid bodies [44, 46]. FGF2/4 stimulates the development of hepatoblasts from cultured ESCs and the generation of mature hepatocytes, whereas HGF plays a supportive role in hepatocyte generation from hepatoblasts. Dexamethasone (glucocorticoid hormone) induces the production of adult hepatocyte-specific proteins. This strategy ensures an 80–90% hepatocyte yield. Recently, Wang et al. established a polymer-modified nanoparticle-based sustained delivery system for growth factors to direct stem cell differentiation into hepatocytes [47]. Their approach can help to overcome the limitations linked with current models and make sure efficient delivery of growth factors to improve ESC differentiation toward a hepatocyte-like lineage.

The final and most important step in this strategy involves isolation of absolute hepatocyte population from a heterogeneous mixture containing other hepatic precursors and immature hepatocytes. Basma et al. used asialoglycoprotein receptor ASGPR1 (hepatocyte-specific cell surface marker) expression based sorting to enrich the pure hepatocyte populations [48]. To enhance the isolation efficiency of hepatocytes based on ASGPR1, fluorescent-labeled or magnet-coated antibodies are further proposed [49]. However, further research is required to be performed to isolate definitive hepatocyte population or to obtain a relatively absolute ratio of hepatocytes from ESCs [50].

Despite their success stories, there are a number of ethical issues concerning the use of human ESCs in liver regenerative medicine [50]. Furthermore, pluripotency of these cells is very difficult to handle leading to an uncontrolled regenerative potential. Above all, putative tumorigenicity associated with transplantation of ESCs proves to be an additional barrier for their clinical application [49–50].

4.2.2. Bone marrow stem cells (BMSCs)

In bone marrow, three different pluripotent cell populations, that is hematopoietic stem cells (HSCs), MSCs, and multipotent adult progenitor cells (MAPCs)/endothelial progenitor cells (EPCs), are present [51]. Peripheral blood, umbilical cord blood, and synovial fluid are additional sources of HSCs and MSCs. HSCs and MSCs can be advantageous cell sources for liver

regeneration as compared to hepatocytes since they can be obtained relatively easily from blood and bone marrow of live donors. Since BMSCs are immune-modulators, a reduced chance of graft rejection is an additional property of these stem cells [47, 51]. In clinical trials, patients with autologous BMSC ($CD34^+$ cell) transplantation had no procedure-related complications and showed improved quality of life [30]. MSCs have proven reliable for treatment of liver cirrhosis in phase I and phase II clinical trials as shown in **Table 1**.

Cell source	Liver cirrhosis	No. of patients	Administration route	Follow-up period	Outcomes/clinical significance	References
Hepatocytes (autologous)	Liver cirrhosis	9	intraportal	10 months in only one patient	Longer survival	[40]
$EpCAM^+$ Fetal liver-SCs	Advanced cirrhosis	2	hepatic artery	12 months	Biochemical and clinical improvement	[74]
	End-stage liver cirrhosis	25	hepatic artery	6 months	Improved liver function and MELD score	[32]
BM-MSCs	Decompensated liver cirrhosis	4	peripheral vein	12 months	Well-tolerated and safe procedure; improved liver function	[75]
	post-HCV liver cirrhosis	20	intrasplenic	6 months	Decreased TBIL, AST, ALT, PT; improved ALB, PC, PT, INR	[76]
Autologous BM-MSCs	Alcoholic cirrhosis	11	hepatic artery	12 months	No significant side effects; histological improvement; improved CP score	[77]
	Liver cirrhosis	9	peripheral vein	6 months	No major adverse effects; improved ALB, CP scores	[78]
BM-MSCs (Differentiated *vs* undifferentiated)	post-HCV liver cirrhosis	10: control 15: treated	intravenous	6 months	Improved MELD score, BIL, ALB, and PC	[79]
UC-MSCs	Primary biliary cirrhosis	7	peripheral vein	12 months	No obvious side effects; decreased serum ALP and GGT	[80]
	Post-HBV decompensated liver cirrhosis	15: control 30: treated	intravenous	12 months	No significant side effects; improved liver function and MELD score; reduced ascites	[81]

Cell source	Liver cirrhosis	No. of patients	Administration route	Follow-up period	Outcomes/clinical significance	References
Autologous MSCs from iliac crest	Decompensated cirrhosis	12: control 15: treated	peripheral vein	12 months	No beneficial effect	[82]
	End-stage liver disease	8	peripheral or portal vein	6 months	No adverse effects; improved MELD and liver function	[83]
Allogenic MSCs	Autoimmune disease-induced liver cirrhosis	26	peripheral vein	24 months	No obvious side effects; improved MELD and liver function	[84]
G-CSF mobilization of CD 34$^+$ BMSCs	Severe liver cirrhosis	40: controls 8: treated	subcutaneous	8 months	No adverse events; improved MELD score	[85]
	Alcoholic cirrhosis	11: control 13: treated	subcutaneous	3 months	Effective CD34$^+$ cells mobilization; increased HGF; induced hepatocyte proliferation	[86]
	Liver cirrhosis	18	subcutaneous	3 weeks	No severe adverse events; no liver function significant modification	[87]
Autologous G-CSF-mobilized cultured CD34$^+$ BMSCs	Alcoholic liver cirrhosis	9	hepatic artery	3 months	No side effects; improved BIL, ALT, AST, CP score and ascites	[88]
PBMCs from G-CSF mobilized PB	Decompensated liver cirrhosis	20: control 20: treated		6 months	No major adverse effects; improved liver function	[89]

EpCAM: Epithelial cell adhesion molecule; GGT: γ-glutamyl transferase; ALT: Alanine aminotransferase; TBIL: Total bilirubin; AST: Aspartate aminotransferase; CP: Child-Pugh; HGF: Hepatocyte growth factor; HCV: Hepatitis C virus; PT: Prothrombin time; ALB; Albumin; PC: Platelet count; INR: International normalized ratio; MELD: Model for end-stage liver diseases; ALP: Alkaline phosphatase; UC-MSC: Umbilical cord blood-mesenchymal stem cells; G-CSF: Granulocyte-colony-stimulating factor; BM-MSCs: Bone marrow-mesenchymal stem cells.

Table 1. Clinical trials of cell-based therapies along with their route of administration, follow-up, and outcomes.

Hematopoietic stem cells originating from bone marrow are efficient stem cell population that migrates to the site of injury and participate in the repopulation of damaged tissue. In liver regeneration, this stem cell population is postulated to contribute based on the cell fusion capability of the BMSCs [52, 53] rather than cellular differentiation. In murine hepatectomy models, BMSCs were found to fuse with hepatocytes, and the resultant hybrid cells were shown to be responsible for triggering proficient liver regenerative reaction [54]. Therapeutic mechanisms of MSCs are reported to be more clear as compared to those of HSCs. MSCs not only reduce

inflammation and fibrosis but they also increase liver regenerative response in a much rapid manner than HSCs [55]. CD34 is reported to be an efficient cellular marker for the isolation of HSCs [30]. However, these cells have showed profibrogenic potential in some cases [56].

Despite wide use in preclinical setting and clinical trials, the BMSCs have to be evaluated extensively for their potential role in liver regeneration before being applied to the wide clinical utilization. Tumorigenicity of MSCs is another constraint that needs to be considered while using this stem cell population in clinical application [57].

4.2.3. Adipose-derived stem cells (ADSCs)

Adipose tissue is another source of MSCs used for hepatic regeneration. ADSCs seem to be pluripotent and have the potential to differentiate into cells of multiple germ lines such as bone, nerve, heart, and adipose tissue. These cells are advantageous over BMSCs because of their higher in vitro proliferation activity and differentiation potential [58]. The sufficient availability of adipose tissue from most patients with no substantial defects renders ADSCs an efficient alternative source of stem cells for liver regeneration [59]. Differentiation of ADSCs into functional hepatocytes involves activation of Wnt/beta-catenin signaling through glycogen synthase kinase 3 inhibitors [60]. Further research is needed to evaluate the potential of this stem cell lineage in liver regenerative setups.

4.2.4. Liver stem/progenitor cells (LSPCs)

Hepatoblasts being bipotent are capable of self-renewal and differentiation into cholangiocytes and hepatocytes. In contrast to ESCs and MSCs, both of which need to go through sequential differentiation to develop into mature hepatocytes, LSPCs have a destined fate. Hence, they carry significant potential to be used in liver regenerative medicine. LSPCs can undergo several rounds of proliferation. These cells have the potential to differentiate into hepatic and biliary cell lineages and to repair the damaged liver tissue [50, 61]. LSPCs are thought to be the cells that do not contribute to the routine liver yields. Instead, they appear in advance stages of liver injury such as primary biliary cirrhosis and nonalcoholic cirrhosis [21]. Many properties of embryonic hepatoblasts are shared by LSPCs. Certain surface markers help in selective isolation of LSPCs via immune selection. They express epithelial cell adhesion molecules (EpCAM) and have been isolated against this surface marker [11] from fetal as well as adult human liver [62]. Differentiation of EpCAM-positive cells can yield both hepatocytes and cholangiocytes [63, 64]. Clinical trials of EpCAM-positive LSPCs are given in **Table 1**.

LSPCs, on the other hand, have certain limitations which hinder their application in liver regenerative medicine. First of all, these cells are present in a very small quantity in the adult human liver making it unproductive to isolate them on the basis of their markers. Our research group had addressed this problem in a recently published study, where BMSCs were differentiated toward oval cell-like cells. These oval cell-like cells were comparable to control oval cells in their efficiency to reduce liver injury [65]. Another major issue associated with LSPCs is their great potential to induce hepatic tumorigenicity. Presently, this is a major limiting factor for

their utilization in liver therapeutics and regenerative medicine. Notably, human liver progenitor cells have been found to be present and contributing in the development of nonalcoholic steatohepatitis in pediatric and adult human patients. They are supposed to be playing fibrogenic role in such cases as reported by Sobaniec-Łotowska et al. [66]. Comprehensive research at preclinical level is required to probe into these issues properly to understand the appropriateness of these cells for clinical trials.

4.2.5. Induced pluripotent stem cells (iPSCs)

The establishment of iPSCs by reprogramming somatic cells through certain transcription factors (Oct-3/4, Sox2, Nanog, c-Myc, Klf-4) has proven a potential new source of stem cells. These cells exhibit properties essential for ESCs and have the potential to differentiate into the derivatives of all three germ layers [67]. However, iPSCs avoid the ethical issues related to ESCs since no human embryo is used for their production [3]. iPSCs being autologous in nature also evade the problem of immune rejection. Although there are unlimited sources for iPSCs generation, to ensure a relatively homogeneous hepatocyte culture, the use of hepatocytes or/and other endodermal cells is recommended. It can play an important role as cells carry an "epigenetic memory" allowing the iPSCs to differentiate toward cells of definitive germ layer [68].

Permanent retroviral integration, a process which was initially used by Takashi and coworkers in 2007 [69] is one of the earliest methods used for iPSCs production. With advancement in the field, it is possible to generate iPSCs without using retroviral transfection. Nowadays, a number of methods such as excisable viral transfection [70], microRNA transfection [71], episomal plasmid transfection [72], and mRNA transfection [73] are being harnessed for the production of functionally efficient iPSCs. Once generated, iPSCs can be directed to differentiate toward definitive endoderm which will differentiate into hepatoblasts and finally into hepatocytes in a sequential manner involving various growth factors, cytokines, and signaling pathways as described previously in this chapter. The resultant hepatocyte-like cells are more like fetal hepatocytes rather than mature hepatocytes, a phenomenon shared by all the stem cell-generated hepatocytes [3]. Although an efficient source of autologous transplantation, iPSCs-derived hepatocytes have certain shortcomings as well.

5. Tissue engineering and liver cirrhosis

Cell-based therapies have shown promising results in the improvement of liver cirrhosis. However, inefficient engraftment of cells due to surrounding conditions of diseased liver results in variable outcomes [3]. Tissue engineering, a recent advancement in liver regenerative medicine, is dedicated in deriving the ways to escape the problems associated with direct cell-based therapies. It mainly focuses on the development of biocompatible scaffolds and extracorporeal liver devices suitable for either in vitro or in vivo applications. Schematic representation of key approaches used for liver tissue engineering is shown in **Figure 3** and discussed in detail with their merits and relevant complications in the following section.

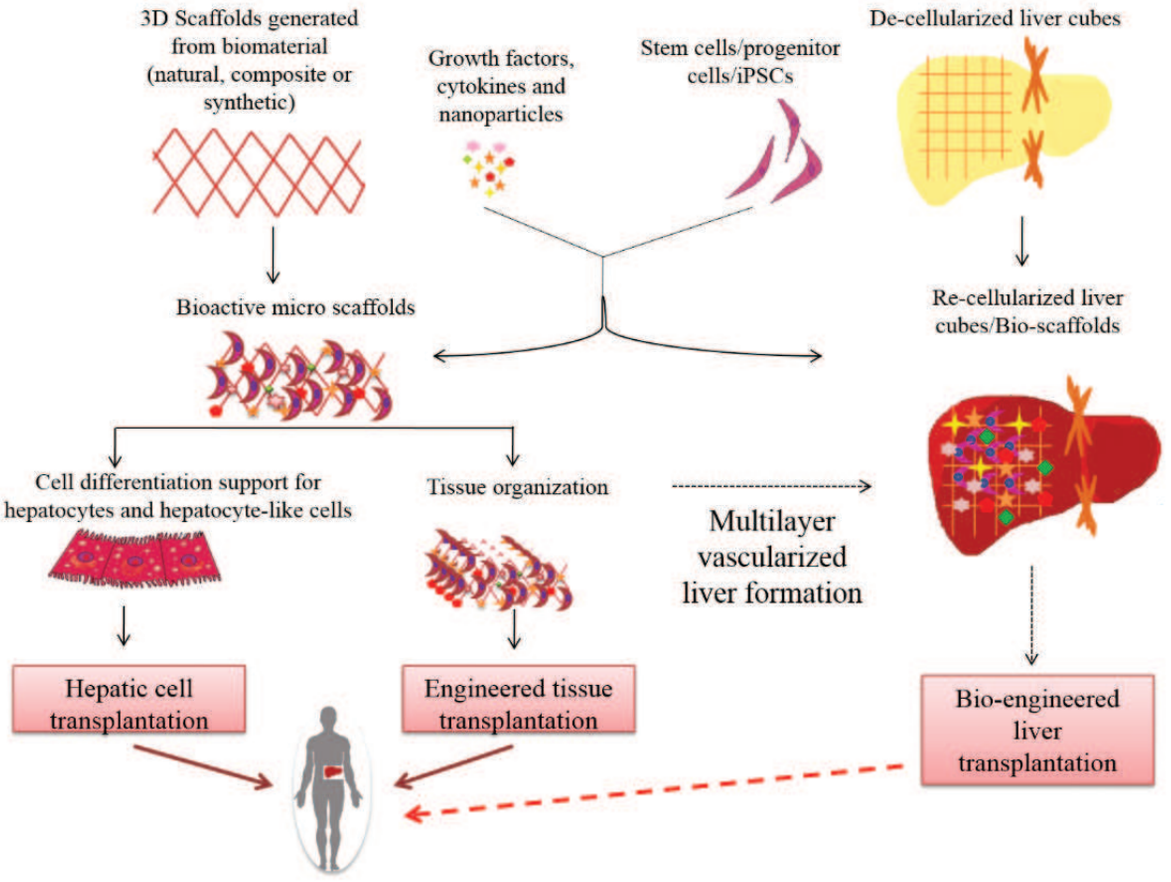

Figure 3. Schematic diagram of liver tissue engineering. Solid lines show the approaches already ongoing whereas dotted lines indicate the proposed mechanisms.

5.1. Generation of bioactive scaffolds

Bioactive scaffolds are those that have the ability to elicit cell growth and differentiation. In modern tissue engineering, bioactive scaffolds are so much advantageous as they mimic the natural ECM environment of the liver. One of the major components of these scaffolds is a structural protein collagen normally found in skin, bone, and cartilage [90]. Collagen highly supports attachment, proliferation, differentiation, growth, and migration of cells. Further, collagen-based bioscaffolds have shown in vitro differentiation of embryoid bodies derived from embryonic stem cell into hepatocyte-like cells [91, 92]. Hyaluronic acid is another important component of the extracellular matrix. It is involved in the regulation of cell proliferation and expansion. The immature and mature hepatocytes of fetal and adult liver cells express surface receptors for hyaluronic acid, that is CD44 [93]. By utilizing this property of hepatocytes, hydrogels consisting of hyaluronic acid and its derivatives are synthesized possessing more adhesive power for hepatocytes. They can retain viability of hepatocytes for 4 weeks [93].

Other natural biomaterials being utilized in the formation of bioactive scaffolds are alginate, chitin, chitosan, silk, matrigel, and sponge. Its best example is silk-fibroin-based microfluidic devices that successfully supported the growth and differentiation of HepG2 cells [94]. Hepatic

organoids and smaller parts of tissues can be grown from porcine hepatocytes on the matrix, consisting of albumin and chitosan (a deacetylated form of chitin) [95]. Scaffold containing chitosan nanofibers associated with the glucose residues showed prolonged metabolic activity of cluster of cells originated from hepatocytes [96]. Hydrogels formed by the natural biomaterials such as alginate and matrigels are more biocompatible and improve the seeding potency of hepatocytes. The basal membranes of murine chondrosarcoma are used for extraction of proteins (laminin, heparan sulfate proteoglycan, collagen type IV) that are used in the formation of matrigels. Hepatocytes initially started to grow in scaffolds containing matrigels into shapeless clusters of cells followed by their implantation in natural organ [97].

However, it has not yet been recognized that which composition would provide the best physicochemical characteristics for defined growth pattern of hepatocytes. Moreover, due to xenogeneic and tumorigenic origin of matrigels, they are not considered good for tissue engineering of liver. Although utilization of natural polymers in three-dimensional (3D) scaffolds creates some histoarchitectural features that help a lot in the generation of cell-to-cell and cell-to-matrix interactions, uncontrollable physicochemical properties, degradability, lack of regenerative ability, and inconsistent mechanical properties halt its clinical implication.

5.2. Synthetic polymers used in liver tissue engineering

In comparison to natural biomaterials used in tissue engineering, synthetic materials provide a wide range of properties and a better control over them. Their biocompatibility and biodegradability can be tuned easily. Scaffolds containing biodegradable polymers facilitate regeneration, transplantation, and degradation of cells on time. Commonly used biodegradable polymers are polylactic acid, polyglycolic acid, polyanhydrides, polyfumarates, polyorthoesters, polycaprolactones, poly- L–lactic acid, and polycarbonates [98].

A synthetic chemical polyglycolate–polylactate used in 3D scaffolds can turn fetal hepatoblasts to mature hepatocytes [99–101]. The main limitations of polyglycolate–polylactate are chemical unpredictability, surface corrosion, and hydrophobicity [102]. However, chemical instability of poly (alpha-hydroxy) acids results in the formation of hydrolysis products, which can induce inflammatory responses. The chemical modification of polymers (e.g. the incorporation of proteins and special bioactive domains) increases the biocompatibility of bioengineered matrices and improves scaffold adhesion properties stimulating cell attachment and migration, thereby, facilitating liver tissue repair [103]. 3D hepatocyte cultures can also be grown successfully in polyurethanes. Polyurethane foams are used to grow hepatocytes and hepatocyte-like cells in bioreactors. Highly functional multicellular structures are formed within the pores of these polyurethane foams [104]. Because of these characteristic polyurethane foams are widely used in 3D scaffolds for the production of bioartificial liver [105].

5.3. Implementation of nanotechnology and microchip devices in tissue engineering

Nanotechnology and microchip devices have tremendous use in liver tissue engineering. Microfluidic devices containing very small volumes of cells, effector molecules, ECM, and so on are used to produce natural biochemical environment around the cells so that they may behave as they do in natural organ [106]. Using the microbioreactors, microcapsule fabrication is done

that leads to the encapsulation of hepatic cells and their precursors. In these special kinds of bioreactors, the regular supply of oxygen, water, and nutrients is ensured and metabolic wastes are eliminated. These capsules are made of polydimethylsiloxane and its derivatives because they are highly permeable to water. The polydimethylsiloxane capsules and microspheres of alginate have showed efficient growth of encapsulated hepatocytes that were seeded on them due to its radical perfusion properties. Due to its remarkable properties, polydimethylsiloxane is a promising tool for bioartificial liver system [72].

To estimate cytotoxic effects of drugs on liver cells, 3D microfluidic cell panels have also been introduced. These panels create the natural environment for cells as they are made up of porous hydrogels and are lined with hepatocytes. These pores are taken as capillaries by the cells. Various pharmacokinetic models are being studied with the help of these panels [107, 108].

Speaking collectively, complex microarchitecture of liver tissues having proper cell to cell interactions and supply of cells with oxygen and nutrients are produced from biologically produced microorgans of liver. These microorgans are produced ultimately from bioactive microscaffolds; 3D hepatocyte panels [109].

5.4. Organ-based regeneration of liver

The development of whole organ using different techniques in tissue engineering is remarkable and this decreases the problems related to shortage of donor organs for transplant and immunosuppression. In order to build a functional liver organ, the first and foremost needed is a scaffold. Among many of the trialed materials for scaffolds, porcine/murine-based scaffolds have proved better. Second, what is needed is the presence of extracellular matrix in the scaffolds to provide the hepatocytes with their niche for their optimal growth and regulation of cellular behaviors [110, 111].

Complete decellularization of native organ is achieved via detergent perfusion for 24–48 hours, in order to get a xenogeneic scaffold. A point that must be mentioned while decellularization is: ECM should not be damaged and it should have under 50 ng double-stranded DNA/mg of ECM to avoid immune rejection [112]. After decellularization, recellularization of xenogenic scaffold with highly functional hepatocytes is done. These cells are obtained either from deceased donor grafts or from partial hepatectomy. However, it is difficult to obtain an appropriate volume of cells. The adult hepatocytes are not considered good for organ regeneration because they show poor in vitro proliferation. Fetal liver cells show high in vitro rate of proliferation but they are not easy to obtain. The human-derived cell lines that show exponential growth in vitro also cannot be used for implantable organs as they pose the threat of metastasis [113, 114]. Porcine hepatocytes remained successful in BAL system but due to immunogenic rejection they cannot be used for organ bioengineering [5]. Human-derived autologous stem cells, that is iPSCs, are capable of producing liver-specific proteins but they produce the albumin at a lower rate than in adult human liver so they are also not a good choice. However, human bone marrow cells are showing promising results in vitro, though they are not yet tested clinically [115].

The recellularization of scaffolds fitted in the tissue cultures of organ chambers is done either by direct parenchymal injections or by single or multistep perfusion in physiological

pressure. As a proof of whole liver decellularization and recellularization concept a rat model was utilized for the proliferation of adult rat hepatocytes. Proliferation was confirmed by different markers. Ninety percent of hepatectomized rat models that were given spheroid tissue-engineered liver showed an increased survival period from 16 to 72 hours. But to their dismay, the rats died of the small-for-size syndrome [116, 117].

Besides facing problem in the selection of most suitable cell lines, another hurdle is to develop a vascular network for the support of cell aggregates [118]. Organ bioengineering offers a hopeful way to get out of complications associated with liver cirrhosis. The best scaffold onto which organ is tissue engineered is a decellularized xenogenic scaffold having intact network of ECM. Studies are being focused on the determination of ideal cell types for humans. Deep research is also going on to find the optimal cell seeding techniques and cell volume required to sustain necessary functions [5].

6. Conclusion

In conclusion, the field of regenerative medicine has taken a successful initiative toward the ultimate solution of end-stage liver diseases. Particularly, the dynamism of various cell-based therapies has arisen much hope and facilitated the development of more challenging tissue engineering. Initially, tissue engineering focused on the use of natural and synthetic scaffolds to grow hepatocytes and develop liver tissues. Currently, much work is ongoing to create liver microorgans to organoids. Crucial aim of future research is to construct whole bioengineered liver. In this regard, the use of decellularized livers has been proposed to create liver organoids leading to the construction of whole bioengineered liver. However, organ bioengineering faces the problems of selection of suitable cell type and appropriate development of a vascular network, which will support cell aggregates. Major challenges associated are the determination of suitable cell type, optimal cell volume, and seeding techniques required to endure essential hepatic functions. The current scenario propels to conduct much more experimental work to successfully construct whole bioengineered liver and its effective clinical applications to replace liver transplantation.

Author details

Asima Tayyeb[1]*, Fareeha Azam[2], Rabia Nisar[1], Rabia Nawaz[4], Uzma Qaisar[1] and Gibran Ali[3]

*Address all correspondence to: asima.sbs@pu.edu.pk

1 School of Biological Sciences, University of the Punjab, Lahore, Pakistan

2 Department of Zoology, University of the Punjab, Lahore, Pakistan

3 University of Texas Health Centre at Tyler, Texas, USA

4 Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan

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