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Cell therapeutic options in liver diseases: cell types, medical devices and regulatory issues

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Abstract Although significant progress has been made in the field of orthotopic liver transplantation, cell-based therapies seem to be a promising alternative to whole-organ transplantation. The reasons are manifold but organ shortage is the main cause for this approach. However, many problems such as the question which cell type should be used or which application site is best for transplantation have been raised. In addition, some clinicians have had success by cultivating liver cells in bioreactors for temporary life support. Besides answering the question which cell type, which injection site or even which culture form should be used for liver support recent international harmonization of legal requirements is needed to be addressed by clinicians, scientists and companies dealing with cellular therapies. We here briefly summarize the possible cell types used to partially or temporarily correct liver diseases, the most recent development of bioreactor technology and important regulatory issues.

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1 General aspects

Cell replacement therapies can be envisioned as a promising alternative to orthotopic liver transplantation (OLT) [1]. The main advantage of cell therapeutic approaches to treat liver disease is their less invasive nature compared to OLT, which allows treatment to be performed repeatedly. Liver diseases that can be addressed by cell therapy are acute liver failure, inherited metabolic disease and end-stage liver disease (cirrhosis). It needs to be emphasized that the conditions and requirements for cell therapy are different for each of the diseases [2]. Up to now, direct hepatocyte transplantation or their use in bioreactors have been used to temporarily support liver function in order to bridge patients until OLT [3, 4]. Cell therapy of end-stage liver disease, however, is more problematic—mainly due to the altered hepatic architecture. During the last few years, researchers have tried to evaluate the efficiency of this approach in rodents with chemically induced liver cirrhosis [5–7]. For this approach they injected different cell types, e.g. rat or porcine hepatocytes [8], syngeneic rat hepatocytes [6] or immortalized rat hepatocytes [7] into the splenic pulp. This intra-splenic cell therapy clearly improved liver function and survival of these animals. In humans with end stage liver disease, however, hepatocyte transplantation showed only limited success [9, 10]. One possible reason might be the fact that cells were delivered into the splenic artery and not into the splenic pulp. This is supported by Nagata et al. showing that the route of hepatocyte delivery strongly influences engraftment and function of cells [5].

Thus, there is still great potential in improving therapeutic success either by improving the cell type used or by optimizing the way of application and the choice of the transplantation site [2]. Beside these matters, culture conditions to maintain and expand various cell types or bioreactors as an artificial organ device are potential ways of improvement. Nevertheless, routine application of primary cells or products based on stem cell-derived technology require strict rules which have been implemented by international regulatory organizations such as EMEA and FDA in the past years.

2 Specific clinical applications

2.1 Hepatocyte-based approaches

Sipe [11] defined cell therapy as the use of living cells to restore, maintain or enhance tissue and organ function. Such a strategy has been demonstrated as an achievable therapeutic approach due to progress in methods of isolation and culture of cells derived from several organs and tissues. As one of the first examples of successful cell therapy, bone marrow transplantation was performed on a laboratory level and in humans [12, 13]. For a long time, clinical hepatocyte transplantation has been recognized as a potential treatment for life-threatening liver disease and the basis for proceeding with clinical trials has been provided by extensive laboratory work in animal models [14, 15]. Lots of efforts have been undertaken to provide high numbers of viable hepatocytes isolated under standards of good manufacturing practice. Hepatocytes have been used either fresh or thawed after cryopreservation with slightly better results obtained for fresh cells. Different sites for implantation have been identified revealing the liver and spleen as the most reliable option, but also the peritoneal cavity was used in patients with fulminant hepatic failure [16]. Cells are mainly infused via the portal vein, the splenic artery or a direct splenic puncture.

Hepatocyte transplantation has now been performed for more than 10 years, mainly in the setting of inherited disorders of liver metabolism (see reviews [15, 17, 18]). The vast majority of these treatments have shown that hepatocyte transplantation may cure or alleviate congenital metabolic diseases of the liver, including Crigler-Najjar syndrome type I (lack of bilirubin conjugation activity due to UDP-glucuronosyl transferase deficiency), familial hypercholesterolemia (low-density lipoprotein receptor deficiency), glycogen storage disease type 1a, urea cycle defects [ornithine transcarbamylase (OTC), argininosuccinate lyase (ASL) and argininosuccinate synthase (ASS) deficiency], and cases of congenital deficiency of coagulation factor VII (overview see Table 1).

Although hepatocyte transplantation in humans yielded only initial but not sustained success rates, it might be a promising alternative to auxiliary liver transplantation [31], since even low amounts of cells (5–10% of the liver mass) can provide sufficient function to correct the underlying

Table 1 Overview of successfully performed hepatocyte transplantations

Disease	Gender	Age	Outcome	Year	Ref.
Alpha 1-antitrypsin deficiency	Male	18 weeks	OLT after 2 years	1997	[19]
	Female	52 years	OLT after 4 years	1998	[20]
Ornithine trans-carbamylase deficiency	Male	5 years	Ammonia level normal after 48 h, died after 43 days	1999	[21]
	Male	10 h	Normal protein intake possible, OLT after 6 months	2003	[22]
Glycogen storage disease type 1a	Female	46 years	Improved for 3 years	2002	[23]
Refsum disease	Female	4 years	Improved for 1 year	2003	[24]
Factor VII deficiency	Male	3 and 35 months	Improved with decreased requirement for recombinant factor VII	2004	[25]
Crigler-Najjar type I	Male	10 years	OLT after 3.5 years	1998	[20]
	Female	8 years	Reduced phototherapy time and substantially decrease in post-transplant serum bilirubin	2009	[26, 27]
			OLT after 11 months	1998	
	Male	9 years	Good liver function under continuous immunosuppression (2 years)	2005	[28]
Urea cycle disorders		3 month to 3 years	Good acceptance of cells in 2 patients/1 child died (follow-up from acute liver failure)	2008	[29]
		1–3 years	Periods of hyperammonemia and clinically relevant crises could be reduced (13 months)/one child died after 3 months from a fatal metabolic decompensation	2009	[30]

metabolic defects. A major drawback for this approach is the number of high quality transplanted cells needed, which have to be freshly prepared or from cryopreserved cell stocks and the need for immunosuppression to prevent the rejection of the transplanted cells.

Fetal liver cells as an alternative to mature hepatocytes may be suitable for overcoming the limitations in engraftment and to allow a functional correction of the disease phenotype. This might be applicable at least in disorders in which a low percentage of cells with a correct genotype are sufficient. Only a few studies have reported modest clinical improvement [16] by using human fetal liver cells in the treatment of acute liver failure. Additionally, ethical issues have to be considered when fetuses from late abortions are used.

2.2 Temporary liver support systems

To provide successful temporary support of the failing organ in end-stage liver disease, the complex metabolic performances of liver cells have to be considered. The aspect of detoxification is addressed by diverse artificial liver support systems that are mainly based on removal of toxins from the blood through mechanical and/or chemical adsorption [32–35]. Although successful removal of plasma toxins could be demonstrated in clinical applications, the therapeutic efficiency of artificial liver support systems is still debated.

To address the aspects of hepatic metabolism and synthesis in addition to detoxification, cell-based liver support systems, also referred to as bioartificial liver support systems, are under development. These systems are intended to use the physiological functions of vital liver cells to replace, support or bridge the function of the failing organ until liver transplantation is available or until organ recovery in case of acute hepatic failure [36, 37].

Several reviews comparing and contrasting the various approaches in clinical practice are available [38–41]. Table 2 gives an overview on bioartificial liver support systems that have been clinically used for extracorporeal liver support. The results of initial clinical trials have shown a tendency towards improvement in biochemical and clinical parameters of patients with liver failure. However, a significant impact on survival and clinical outcome could not be shown clearly due to the lack of randomized clinical efficacy studies.

One major limitation of the existing bioartificial liver support systems can be seen in the cultivable cell mass, which in most systems is only marginally above the minimal cell mass needed for sufficient organ support. It is assumed from liver partial resection studies that 10–20% of the mass of an adult human liver, corresponding to 150–300 g of vital and fully functional cells are required [53]. However, in case

Table 2 Bioartificial liver support systems used in clinical trials

Bioreactor technology	Cell type	Way of perfusion/oxygenation	Clinical studies: outcome	Ref.
Hollow fiber-based bioartificial liver (ELAD®)	Human hepato-blastoma cell line (C3A)	Indirect perfusion via diffusion/oxygenation through perfusate	Phase I study: safety of system shown, Improvement of blood parameters, no significant increase of survival versus control	[42–44]
Hollow fiber-based bioartificial liver perfused with whole blood (BLSS)	Porcine hepatocytes	Indirect perfusion via diffusion/oxygenation through perfusate	Phase I study: safety of system shown, improvement of blood parameters	[45, 46]
Hollow fiber-based bioartificial liver with hepatocytes attached to dextran microcarriers (HepatAssist)	Cryo-preserved porcine hepatocytes	Indirect perfusion via diffusion/oxygenation through perfusate	Prospective, randomized multi-center study: improved survival, although not statistically significant	[47, 48]
Amsterdam Medical Centre Bioartificial Liver Device (AMC-BAL)	Porcine hepatocytes	Direct cell compartment perfusion/ oxygenation through hollow fibers	Phase I study: improvement of clinical and blood parameters	[49]
Radial Flow Bioreactor	Porcine hepatocytes	Direct cell perfusion/oxygenation through perfusate	Phase I study: improvement of clinical and blood parameters	[50]
Hollow fiber-based bioartificial liver with integral oxygenation (MELS)	Porcine or human liver cells	Cell supply possible via perfusion or diffusion/direct membrane oxygenation	Phase I study with porcine hepatocytes Pilot study with human hepatocytes: clinical safety shown, improvement of clinical and blood parameters in some patients	[3, 51, 52]

of using porcine cells, the needed cell mass might be distinctly higher due to the different metabolic capacity of pig hepatocytes compared with human hepatic cells.

Another limitation is the present unavailability of an efficient and safe cell source, as mentioned above. Most systems are using porcine liver cells, which is associated with a potential risk of xenogenic virus transfer, in addition to possible immunological reactions. In addition, the well-known species-dependent differences in metabolic performances between pigs and humans cause functional discrepancies [54]. Human hepatoma cells, used in the ELAD system, have the advantage of good availability and practicability, but alterations in cell metabolism due to cell transformation lead to functional impairment. Primary human hepatocytes currently represent the gold standard with respect to human-specific functionality and clinical safety [54, 55]. However, the limited availability of primary human hepatocytes at sufficient quality and quantity for clinical application make them unsuitable for the performance of larger clinical studies. Therefore, there is a need to develop alternative cell sources with improved availability and stability.

Both transplantation of hepatocytes in the setting of acute liver failure and the use of bioartificial liver devices can currently only be seen as a method to improve the metabolic condition of extremely ill patients prior to transplantation. Furthermore, liver cell transplantation and bioartificial devices are still an experimental approach, while orthotopic liver transplantation is a well-established life-saving procedure. Therefore, an ethical conflict arises when assigning high quality organs and tissues to cell therapy programs or to organ transplantation centers while many patients still die on the waiting list.

2.3 Stem cell-based approaches

Additional sources of cells might be necessary, such as reversibly replicating hepatocyte cell lines [56] or stem and progenitor cells capable of generating hepatocyte-like cells [14, 57–62]. There are three characteristics which make stem cells different from other cell types: (i) stem cells are un-specialized but (ii) pluripotent cells which have the ability to (iii) indefinitely renew themselves. The ability of indefinite self-renewal offers great perspectives for cell therapeutic approaches, which strongly depends on cell sourcing. Stem cells are considered to be potential instruments to address the major problems of liver cell therapy developments: the limited availability of suitable cells and the risk of immunological reactions following clinical cell therapy [63]. To date, scientists primarily work with two kinds of stem cells, namely embryonic stem cells (ESCs) and adult (or somatic) stem cells (SSCs). The use of either embryonic or adult stem cells as cell sources in regenerative medicine is the subject of intense research and controversy. The decision for one or the other may largely depend on the specific clinical application and has to be made after direct experimental comparison. Despite major progress has been made in identifying and evaluating appropriate stem cell sources, there have been no clinical trials using stem or progenitor cells to treat liver diseases in human patients because protocols have not been transferred to clinical applications.

With respect to potential clinical use, adult stem cells provide several advantages over ESCs. Adult stem cell therapy can be performed with autologous cells taken from the patient to prevent clinical rejection and to avoid immunosuppressive therapy. The risk for tumor development from adult stem cells is likely to be lower than with ESCs. On the other hand they typically generate the cell types of the tissue in which they reside. However, a number of experiments have proven a great plasticity of adult stem cells to overcome this limitation.

Several approaches to culture, expand and differentiate adult liver stem cells have been described [64] and great effort has been made to elucidate the underlying processes that stem cells may thereby undergo (overview see Table 3). The phenotypes of progenitor cells from fetal or adult liver were characterized with respect to their marker expression profile in vitro and in vivo [62, 65–67]. The role of cytokines, growth factors, hormones, extracellular matrix proteins and co-culture with non-parenchymal “feeder cells” has been studied extensively [68, 69]. Numerous articles have reported about the generation of hepatocyte-like cells from different types of extra-hepatic stem or precursor cells, e.g. peripheral bone marrow cells (PBMCs) cultured in the presence of IL-3, M-CSF and following FGF-4 [70, 71], the pancreatic exocrine cell line AR42J-B13 treated with dexamethasone [72, 73], adipose or liver tissue-derived mesenchymal stem cells (MSCs) treated with EGF, HGF, FGF-1, FGF-4, OSM and dexamethasone in varying combinations [74, 75]. Other approaches focus on suppressing the asymmetric cell kinetics program resulting in senescence behavior in vitro in stem cells of many adult somatic tissues [76].

Studies on adult liver progenitor cells in multi-compartment bioreactors suggest that cell maintenance at high densities in a complex microenvironment allowing physiological signal exchange and cell communication supports cell proliferation and tissue regeneration in vitro [87, 88]. Furthermore, improved hepatic maturation of human fetal hepatocytes was observed in perfused 3D bioreactors [89]. However, the mechanisms underlying liver stem cell propagation in vitro are not completely clear, and directing controlled differentiation in vitro is still a problem. In addition, a scale-up of culture methods is needed to attain sufficient cell numbers for clinical use.

In contrast, human ESCs could provide an unlimited cell source for cell therapy due to their pluripotency and

Table 3 Adult progenitor/stem cells for liver cell transplantation

Cell source	Characterization	Transplantation	Ref.
Bone marrow progenitor	Hepatocyte marker profile by RT-PCR and IF; urea and albumin secretion; Phenobarbital inducible CYP450; Glycogen storage; LDL uptake	No	[77]
Rat bone MSC line	CD profile by flow cytometry; hepatocyte marker profile by RT-PCR and IF	Improved survival of rats with subtotal hepatectomy	[78]
MSC-like cells (liver)	CD profile by flow cytometry; urea and albumin secretion; Glycogen storage; hepatocyte marker profile by RT-PCR and IF	uPA(+/+)-SCID and SCID	[75]
Adipose-derived MSCs	CD profile by flow cytometry; hepatocyte marker profile by RT-PCR and IF	Pfp/Rag2$^{-/-}$ mice	[79, 80]
Mono-nuclear cells	CD profile by flow cytometry; hepatocyte marker profile by RT-PCR and IF; urea, glucose and albumin secretion; phase I and II drug metabolizing enzyme activities; NH_3Cl metabolism	Injection of cells improved survival after subtotal hepatectomy in Wistar rats	[71, 81, 82]
Fibroblasts	CD profile by flow cytometry; urea and albumin secretion; Glycogen storage; hepatocyte marker profile by RT-PCR and IF	Hepatectomized SCID mice	[83]
Pancreatic cell line	Hepatocyte marker profile by RT-PCR and IF; albumin secretion; phase I and II enzyme expression by RT-PCR and IF	No	[84]
Pancreatic cell line	Hepatocyte marker profile by RT-PCR and IF; expression of phase I and II drug metabolizing enzymes; lipid accumulation upon insulin treatment	No	[85]
Human small hepatocytes	Hepatocyte marker and cytochrome P450 profile by RT-PCR	No	[86]
Human liver progenitor cells	CD, stem cell marker profile by flow cytometry; hepatocyte, biliary marker profile by RT-PCR and IF, albumin expression	Monocrotalin, hepatectomized Pfp/Rag2$^{-/-}$ mice	[62]

Table 4 Embryonic and iPS cells for liver cell transplantation

Cell source	Characterization	Transplantation	Ref.
Human ESCs	Stem cell and hepatocyte marker profile by RT-PCR and IF; a-FP promotor reporter assay;	No	[111]
Human ESCs	Stem cell and hepatocyte marker profile by RT-PCR and IF; Cytochrome P450 and albumin expression by Western blot	Retorsin injected SCID-NOD mice	[93, 112]
Monkey ESCs	Hepatocyte marker and cytochrome P450 profile by RT-PCR	No	[113]
Mouse iPSCs from MEFs	Stem cell and hepatocyte marker profile by RT-PCR and IF; Cytochrome P450 expression by RT-PCR	No	[114]

proliferation capacity. There is extensive research on human ESCs as resource for cell therapeutic approaches [90–92] and successful hepatic differentiation of human ESCs has been shown [93–95]. However, the yield and purity of hESC-derived hepatic cells is still not sufficient. In addition, the availability of undifferentiated ESCs for differentiation is currently limited by the technically challenging and labor-intensive methods required to propagate the cells in their undifferentiated state without producing karyotype abnormalities. To prevent tumor formation caused by the cells, improved methods are needed to regulate proliferation, to control the maturation process, and to purify cell preparations before transplantation, thus guaranteeing safety in clinical application. Furthermore, the use of human embryonic cells is highly controversial from a legal and ethical point of view. Major ethical issues are the generation of human ESCs from embryos fertilized in vitro, the moral status of the embryo, and the acceptability of using such derived cells for therapeutic purposes.

In the future, autologously induced pluripotent stem (iPS) cells could replace human ESCs as a source of donor cells and therefore omitting the ethical debate about using ESCs (see Table 4). Recent reports demonstrated the potential to create pluripotent stem cell lines while reprogramming postnatal cells by transferring only a few genes [96–98]. Takahashi and Yamanaka [99] reported about the generation of iPS cells from mouse embryonic fibroblast cultures by transfection of transcription factors Oct4, Sox2, c-Myc and Klf4. The cells had similar characteristics as ESCs with respect to morphology, proliferation and teratoma formation. The first iPS cells from adult human cells were achieved by retroviral transfection of Oct4, Sox2, c-Myc and Klf4 [98, 100] or Oct4, Sox2, Nanog and Lin28 [101]. Furthermore, several groups tried to generate iPS cells by retroviral gene transfer of the aforementioned factors from various types of cells, e.g. mouse and human (embryonic) fibroblasts [102–104], adult dermal fibroblasts [105–107], neural stem cells [108, 109], human keratinocytes [110] and human blastocysts [98, 99]. Induced pluripotent stem (iPS) cells might have a promising future in cell therapy, but there are still many issues that have to be investigated before these cells can be used in clinical settings, e.g. cancerogenicity and safety issues of retroviral gene transfer.

Without doubt, the wide availability of human hepatocyte-like cells would be considered a major breakthrough and may open new perspectives for the treatment of liver disease. However, these approaches are conceptual and still far from clinical application. Furthermore, there is a crucial need for better characterization of generated human hepatocytes, hepatocyte-like cells or precursor cells.

2.4 Bioreactor technologies for extracorporeal liver support

To overcome the limitations of existing extracorporeal liver support systems, various bioreactor technologies have been developed to improve cell maintenance in vitro. For example, a coaxial hollow fiber-based bioreactor with integral oxygenation [115] was successfully tested in vitro with rat hepatocytes. An alternative approach was introduced with the construction of flat membrane bioreactors allowing integral oxygenation [116, 117]. In vitro experiments and in vivo animal studies using porcine hepatocytes showed liver-specific functions and successful outcome in treating fulminant hepatic failure using the flat sheet bioreactor technology [118]. A prolonged survival time was observed in pigs with complete liver ischemia treated with a bioartificial liver device that uses integral oxygenation and a spirally wound non-woven polyester matrix for hepatocyte culture [119]. Further technological approaches are based e.g. on micro-patterned borosilicate wafers [120], biodegradable polymers constructed via 3D printing [121], micro-fabricated constructions by ion etching of silicon wafers [122, 123], hydrated polyester fibers coated with autologous biomatrix [124] or non-woven polyurethane matrix with integral oxygenation [125]. These systems showed that optimization of conditions within the cellular micro-environment, like plasma flow rates in cell chambers and oxygen tension profiles, has a significant impact on specific

biological performances in terms of metabolic activity and substance production; however, extension of these designs toward clinical dimensions has been a challenge.

2.5 Multi-compartment bioreactor technology for high-density perfusion cell culture

We focus on bioreactor developments that address the cellular needs of 3D tissue density conditions [126, 127]. A major feature of the developed technology is the option of scaling up the technology from small analytical devices for research purposes towards large systems for the expansion of cells and for their use in clinical bioartificial liver support.

The multi-compartment bioreactor (see Fig. 1) consists of two independent bundles of hollow fiber microfiltration membranes for the transport of culture medium (medium compartments), inter-woven with one bundle of hollow fiber oxygenation membranes for the transport of oxygen, carbon dioxide, and/or other gases (gas compartment). The inter-woven fibers form a 3D scaffold for the cells residing in the interstitial space between the capillaries (cell compartment) [126, 127]. Thereby, the four-compartment design allows integral oxygenation of the cells with efficient transfer of nutrients to the cells and removal of waste products from the cells. In addition, the construction principle allows scale-up of the technology from analytical prototypes for less than 1 g of liver cells up to clinical prototypes that accommodate 400–800 g of primary porcine or human liver cells.

The bioreactor is integrated into a perfusion device with separate pump systems for medium circulation and medium substitution, a heating unit for maintaining the temperature of the bioreactor chamber, and a gas mixing unit for the regulation of O_2/CO_2 perfusion.

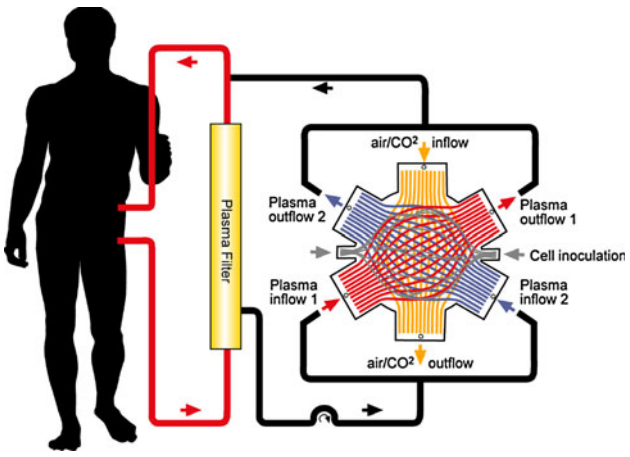

Fig. 1 Extracorporeal liver support system based on a multi-compartment bioreactor technology for high-density perfusion culture of liver cells

Histological and immunohistochemical studies showed spontaneous organization of parenchymal and non-parenchymal liver cells to parenchyma-like cell aggregates between the capillaries (see Fig. 2). Structures resembling liver sinusoids as well as bile-duct line channels were observed in cultures of primary porcine or human liver cells [87, 128]. In addition, maintenance of the differentiated functions of primary liver cells was demonstrated over a period of several weeks [129, 130]. Interestingly, morphological and biochemical signs of cell recovery and regeneration were observed in bioreactors with liver cells obtained from preservation-injured organs [88, 131].

The bioreactor has been successfully used in a device to provide extracorporeal liver support [3]. In a pilot study in Berlin, Germany, the device with primary human liver cells harvested from organs explanted for transplantation but subsequently discarded due to steatosis, cirrhosis or mechanical injury. The results from this study demonstrate the feasibility of employing primary human liver cells in clinical applications with specific bioreactor systems [132]. However, the limiting issue of liver support remains the availability of primary human cells [54]. Although an attractive technology with therapeutic potential is available, the availability of the cell source will therefore be crucial to enable further clinical studies.

Bioengineering research with the aim to further optimize bioartificial liver support bioreactors may consider learning from biomechanical aspects of mass exchange in the natural organs. Unfortunately, this is particularly complex in the liver since this organ exhibits not only arterial and venous capillaries and the connecting sinusoids but also a low-pressure and high-volume portal-venous capillary system leading into the sinusoids in addition to the arterioles.

While the microanatomy of the vascular capillary system of the human liver is well characterized, the physiology of the microvascular perfusion has so far been studied less intensely. Innovative bioreactor technology platforms, however, should consider the available knowledge on the perfusion environment of hepatocytes in the natural organ and consequently try to mimic these conditions. Importantly, the physiology of blood perfusion and plasma exchange between the liver's sinusoids and the hepatocytes demonstrates forces that contribute to an extremely high mass exchange from blood plasma via the sinusoidal fenestrations to the hepatocytes and back [133–135]. An interesting component of mass exchange in the natural liver is the "massaging" of the sinusoids by circular stellate cell to cell arrangements around the sinusoids in the space of Disse [136], resulting in a change of the vascular tonus undulation and consequently in resistance forcing plasma filtration towards the hepatocytes and back. Additional mechanical forces are generated by the heart pump pulsation, the liver capsule pressure changes in the rhythm of the

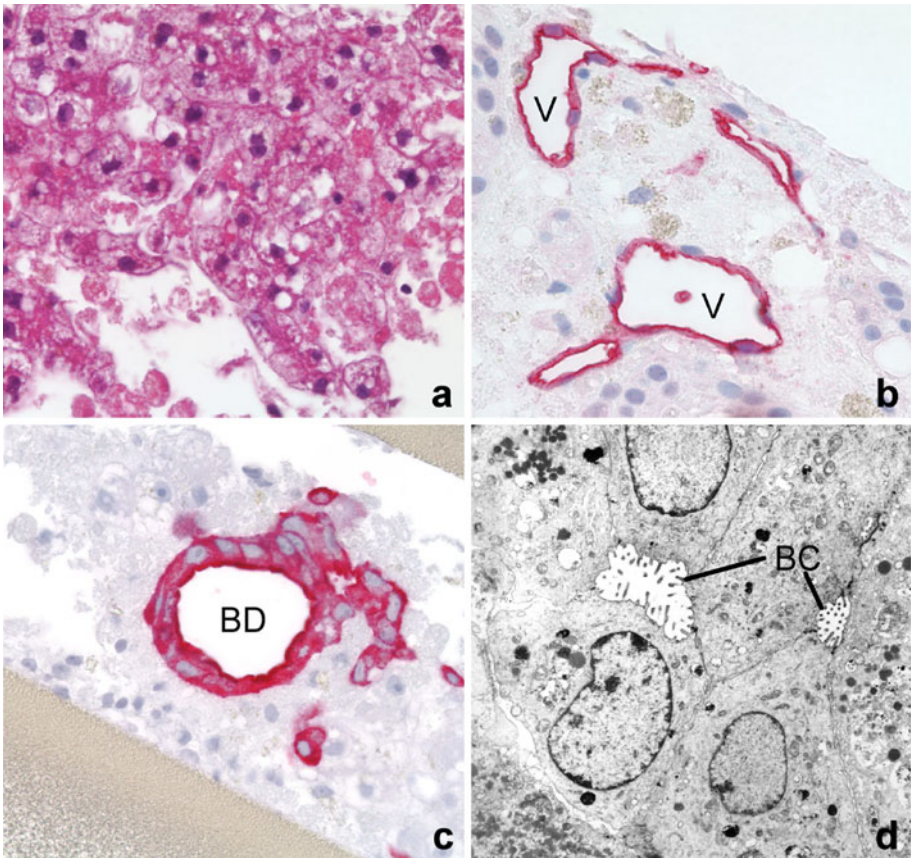

Fig. 2 Re-organization of primary human liver cells in 3D bioreactors. **a** Hepatocyte aggregates (albumin staining), **b** vascular channels (V) lined by endothelial cells (CD 31 positive), **c** biliary-like ducts (BD; CK 7 positive), and **d** bile canaliculi (BC) between adjacent hepatocytes (electron microscopic picture)

lung/diaphragm/abdomen breathing movements, and the regulation of sinusoidal perfusion [137], again forcing plasma filtration via the sinusoidal fenestrations [138] into the space of Disse and back. Since all these aspects enhance plasma mass exchange around hepatocytes in the natural liver, their consideration in the development of the next generation of bioreactors may result in significant mass exchange improvements of such bioartificial systems.

2.6 Regulatory frameworks

In the United States, the FDA has to approve clinical trials of cell-based therapies performed [139]. Similar regulatory bodies will need to approve trials in other parts of the world [140]. In the current state of regulations relevant to stem cell-based products, there is a mismatch between traditional categories (drugs and medical devices) that continue to be used in these regulations, and novel products (like tissue engineering) that do not fit neatly into any of these categories. Recent developments in Europe have addressed this problem, most notably the "Guideline on Human Cell-based Medicinal Products" (EMEA/CHMP/410869/2006) and a new regulation for 'Advanced Therapy Medicinal Products' (Directive 2009/120/EC), which has taken effect in 2009. The latter is an important attempt to provide a consolidated framework for regulatory requirements applicable to novel types of cell products. Its development raised significant issues about the definition of product categories, ethical concerns regarding the cell source, and the application of regulatory requirements to small-scale production. A new Committee for Advanced Therapies will assess products and provide a draft opinion to the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMEA), which will then issue the product authorization [141, 142] (see Fig. 3). Donation, procurement, and testing of human cellular or tissue source materials and the conduct of clinical trials must furthermore comply with existing directives on those subjects (http://www.ema.europa.eu/pdfs/human/cat/57113409en.pdf). Currently, over 40 clinical trials using stem cells in regenerative medicine applications within the EU are being performed under review of the EMEA.

The Committee for Advanced Therapies (CAT) prepares a draft opinion on new Advanced-Therapy Medicinal Products (ATMP) in accordance with Regulation (EC) No. 1394/2007 for the CHMP. The latter one is responsible for answering all questions concerning medication for human use for the EMEA. The CHMP was established by Regulation No. 726/2004 for the authorization and supervision of medicinal products for human and veterinary use and in

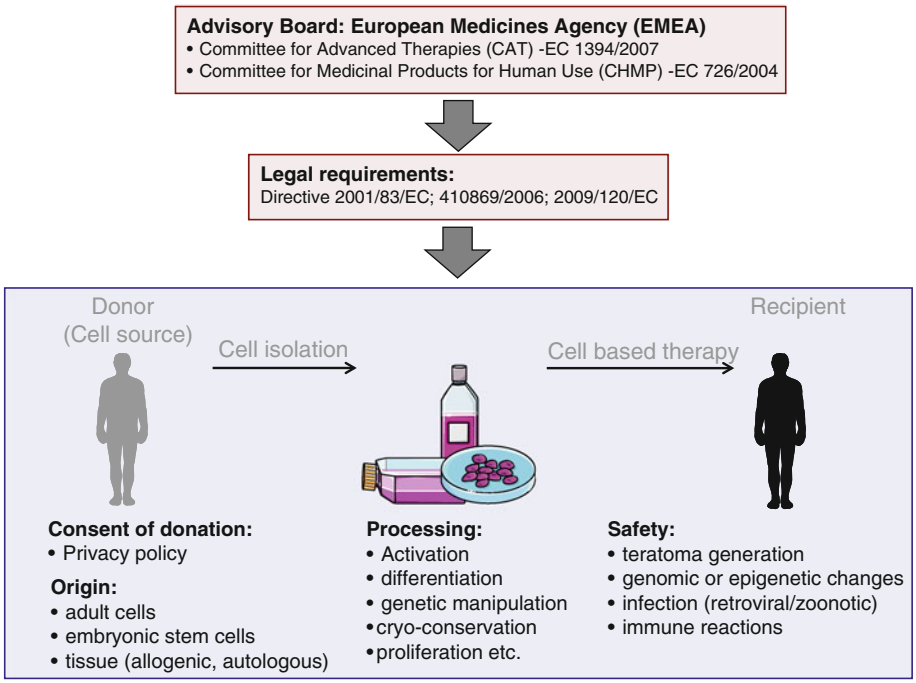

Fig. 3 Regulatory frameworks for therapeutic options in liver diseases

accordance with the Guideline EMEA/CHMP/410869/2006 which regulates development, manufacturing and quality control of cell-based medicinal products. Directive 2009/120/EC defines the use of advanced therapy medicinal products and amends Directive 2001/83/EC related to medicinal products for human use in general.

2.7 Safety issues

There are several issues that relate to safety, efficacy and quality of cell-based products. Some of these are shared with other biological products, whereas others are more specific to stem cell-based products. Safety risks include the potential for tumor formation, e.g. teratoma generation (particularly when pluripotent stem cells are used), possible genomic or epigenetic changes and infection (including retroviral or zoonotic infections), as well as potential immune reactions. In addition, when cells are administered directly, they might not always migrate or differentiate as desired—either of which could create a risk of harm, in addition to compromising the effectiveness of the treatment. Although safety is of utmost concern, this risk will have to be weighed against the potential benefits of the therapy being applied [143].

2.8 Ethical issues

In the past, the main ethical debate was dominated by the controversies on human embryonic cells and therapeutic cloning [144–146]. The lack of ethical consensus about the use of human embryos suggests that these issues are best left open for national debate, and if necessary, for separate legislation at the national or local level, while the regulation of product safety, efficacy and quality remains as inclusive and neutral as possible.

Nevertheless a variety of further ethical aspects has to be taken into account and is discussed in the field of tissue engineering [147, 148]. Using human tissue for cell generation goes hand in hand with an old debate on informed consent of the donor and how far the information about the future use and application should go [149].

Furthermore, ownership of the tissue represents a constant ethical conflict in the context of free and unpaid donation in contrast to the commercialization of cell based therapies from the legal point of few, which can only be overcome by a high degree of transparency through all steps of tissue collection, procurement, allocation and adding value in the development of new cell-based therapies (i.e. http://www.htcr.org).

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