Advanced Drug Delivery Reviews 140 (2019) 51–67

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Microphysiological systems meet hiPSC technology – New tools for disease modeling of liver infections in basic research and drug development

Martin Raasch [a,b], Enrico Fritsche [c], Andreas Kurtz [c], Michael Bauer [b,d], Alexander S. Mosig [a,b,*]

[a] Institute of Biochemistry II, Jena University Hospital, Germany
[b] Center for Sepsis Control and Care, Jena University Hospital, Germany
[c] Charité - Universitätsmedizin Berlin, Berlin-Brandenburg Center for Regenerative Therapies, Germany
[d] Department of Anesthesiology and Intensive Care Medicine, Jena University Hospital, Germany

ARTICLE INFO

Article history:
Received 10 May 2018
Received in revised form 1 June 2018
Accepted 12 June 2018
Available online 15 June 2018

Keywords:
Hepatocyte like cells (HLC)
Viral liver infections
Malaria
Inflammation
Stem cells
iPSC differentiation
Immune response
Drug screening

ABSTRACT

Complex cell culture models such as microphysiological models (MPS) mimicking human liver functionality *in vitro* are in the spotlight as alternative to conventional cell culture and animal models. Promising techniques like microfluidic cell culture or micropatterning by 3D bioprinting are gaining increasing importance for the development of MPS to address the needs for more predictivity and cost efficiency. In this context, human induced pluripotent stem cells (hiPSCs) offer new perspectives for the development of advanced liver-on-chip systems by recreating an *in vivo* like microenvironment that supports the reliable differentiation of hiPSCs to hepatocyte-like cells (HLC). In this review we will summarize current protocols of HLC generation and highlight recently established MPS suitable to resemble physiological hepatocyte function *in vitro*. In addition, we are discussing potential applications of liver MPS for disease modeling related to systemic or direct liver infections and the use of MPS in testing of new drug candidates.

Contents

* Corresponding author at: Center for Sepsis Control and Care, Jena University Hospital, Germany.
E-mail address: alexander.mosig@med.uni-jena.de (A.S. Mosig).

https://doi.org/10.1016/j.addr.2018.06.008

1. Introduction

The human liver is the largest organ of the body (around 1.5 kg in a 70 kg human) and has a multitude of functions, including metabolism of carbohydrates, proteins and lipids but also clearance of toxins and pathogens. Approximately 70%–80% percent of all liver cells are parenchymal cells known as hepatocytes. Non-parenchymal cells (NPC) account for about 40% of total liver cells and include hepatic stellate cells, Kupffer cells (KC) and liver sinusoidal endothelial cells (LSEC) that shield hepatocytes from the bloodstream and blood-borne pathogens. *Ex vivo* it has been shown that NPCs are central modulators of hepatocyte biology and a prerequisite of a proper hepatocellular function *in vitro* [1]. LSEC form the vascular lining of liver sinusoids and represent 15 to 20% of liver cells but only 3% of the total liver volume [2]. LSEC act as a specialized type of endothelial cells that is characterized by the presence of fenestrae (small openings) with a diameter up to 100 nm, making them the most permeable endothelial cells of the mammalian body.

Under physiological conditions components of the ECM likely prevent a passive diffusion of pathogens into the space of Disse [3]. LSEC are involved in clearance of endotoxins and bacteria, and control migration of leukocytes into the liver. The integrity of the endothelial lining of the liver sinusoid is thus fundamental for a coordinated host defense during infection [4]. LSEC represent the major cell type responsible for elimination of connective tissue molecules from the circulation with the highest endocytic uptake rate in the body [5–7]. In contrast to KC that function as a large scavenger cell population and phagocytose particles beyond 200 nm, LSEC are highly efficient for uptake of circulating antigens [8]. KC function primarily as a large scavenger cell population that is localized in the liver sinusoids to phagocytose cell and microbial debris and even gut-derived microbial microorganism that reach the liver within the bloodstream [9–11]. This scavenger function of myeloid cells serves as a firewall function to the liver for clearance of bacteria from the bloodstream that have escaped elimination by gut-associated myeloid cells [12]. Within the liver, KC represent 15% of total liver cells and almost 80–90% of all tissue macrophages in the human body [13]. Macrophages orchestrate acute inflammation, host defense and resolution of inflammation. In the course of infection macrophages are a major source of inflammatory cytokines including interleukin (IL)-1α, tumor necrosis factor (TNF) and IL-6. These cytokines mediate coordinated changes in the transcriptional activity of hepatocytes to limit tissue injury and to secrete meditators of host defense [14]. IL-6 acts directly on hepatocytes and drives acute phase protein production in hepatocytes [15, 16], but also reduces synthesis and release of albumin, transferrin, and fibronectin [17]. These changes are counter regulated by HGF which plays a key role in liver growth and regeneration [18]. However, KC are also involved in maintaining liver tissue homeostasis and regeneration. KC possess a remarkable functional diversity and the ability to rapidly respond to changes in the liver microenvironment. KC activation pattern has been classified in different polarization stages that represent a transient continuum. In this simplified model, the inflammatory M1 polarization and the regenerative M2 polarization state are the hallmarks of two diametric extremes of macrophage activation. In general, the M1 polarization of KC is associated with release of pro-inflammatory cytokines, host defense but also cell death in hepatocytes. In contrast, M2 polarization contributes to tissue repair and promotes the re-establishment of tissue homeostasis [1]. Within the sinusoid blood-borne pathogens are taken up by KC and dendritic cells (DC) and presented to surrounding subsets of T cells [19]. The cross-presentation of pathogen derived antigens thereby triggers a complex local immune response to clear invading microorganisms. Pathogens are detected by pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) expressed not only by Kupffer cells and hepatic DCs, LSECs, hepatic stellate cells but also hepatocytes [20–22]. Hepatocytes are thus also active participants in innate immune response to infection by either host defense or induction of programmed cell death [23]. The immune system of the liver has the ability of a strong innate immune response with a sufficient immune surveillance to clear infectious microorganisms. Conceptually, a dose dependent tolerance to different classes of pathogens including of viral [24], protozoan [25, 26] or bacterial origin [27] has been discussed as an important mechanism in preserving fitness of the host during infection [28]. However, dysregulation of the immune response, *i.e.* during sepsis, is also associated with detrimental immune tolerance towards pathogen associated molecular pattern and adverse effects on pathogen clearance (PAMP) [29]. In this context, NPC in the liver create a system of checks and balances that enables maintenance of tissue homeostasis under physiological conditions as well as a tailored immune response to clear invading pathogens during infection. Moreover, acute on-chronic-liver failure, *i.e.* the acute deterioration of liver function in a patient with chronic liver disease, specifically cirrhosis reflects a prime example, how comorbidities affect the course of sepsis. A dysregulated host response to infection, in particular a hyperinflammatory reaction is characteristic for these patients and propagates deterioration of hepatic and extrahepatic organ dysfunction as well as death [30].

Complex cell culture models mimicking organ physiology and infectious disease related alterations of its microenvironment are in the spotlight of basic research as alternative to conventional cell culture and animal models. Promising techniques like microfluidic cell culture, sophisticated 3D scaffold arrangements or micropatterning by 3D bioprinting are gaining increasing importance for such models to address the needs for more predictivity and cost efficiency. These novel approaches aim to improve the *in vitro* cellular environment by providing clues to generate complex microenvironments to resemble the human physiology more closely. To resemble liver-specific function with an accurate emulation of the organ-specific microenvironment, the source of cells used in MPS has to be carefully considered.

2. Cell sources for modeling liver function

2.1. Primary human liver cells and hepatic cell lines: Status quo

To date, most of the MPS rely on tissue engineering strategies that involve the use of immortalized cell lines derived from malignant tumors or primary cells from patients. Primary human liver cells are regarded as the gold standard. They closely resemble hepatic metabolism *i.e.* protein synthesis and secretion, carbohydrate turnover, hepatic transporters activity as well as drug metabolism. A variety of isolation procedures exists, most of them based on mild collagenase perfusion with the benefit of a step-wise collection of all cell types from one tissue sample. However, *in vitro* primary human hepatocytes (PHH) tend to rapidly lose their phenotype and functionality. In a two-dimensional monoculture, primary hepatocytes show a loss in CYP-dependent

monooxygenases activities [31], significant down-regulation of phase I and phase II enzymes, stress-related up-regulation of acute-phase-response enzymes as well as delocalization of transporter proteins within 24 h of cultivation [32].

This observation could be most likely explained by the oxidative stress that PHH receive during cell isolation and collagenase treatment [33]. Additionally, loss of cell-cell and cell-matrix contacts also impairs hepatocyte polarization [34] and contributes to subsequent cell dedifferentiation. Further, inter-individual donor-related variability of PHH, that are often additionally primed by preexisting morbidities and medications of the donors, as well as limitations in the availability of sufficient donor material makes it often difficult to source enough cells on a routine basis. Furthermore, cell isolation from primary tissue requires complex logistics and well trained personal contributing to high costs for the supply of PHH.

To overcome these PHH specific limitations, a number of different human hepatoma-derived cell lines have been established. Major advantages of hepatoma-derived cell lines are their ease of use, simple logistics and cost-effective maintenance. However, those benefits are often outweighed by a number of other limitations. Most cell lines are derived from carcinoma and are therefore primed in a disease-like state. Further, cell lines have a limited biological relevance compared to primary cell types, due to defects in signaling pathways and cellular dedifferentiation or transformation of cell lines by viruses. For example, Huh7.5 cells are often used to study Hepatitis C virus (HCV) replication, but are not ideal to study virus-host interaction due to defects in the retinoic acid-inducible gene 1 pathway [8]. Even within one cell line, differences occur due to decades of usage in different settings [35]. Consequently, cell lines are less suitable for disease modeling and pharmaceutical screening purposes and data obtained from these cells should be interpreted with caution.

2.2. Stem cells provide promising alternatives to primary cells and cell lines

Human stem cells have already been frequently used in *in vitro* models and can be divided into embryonic stem cells (ESCs) and hiPSCs according to their differentiation potential. ESCs are pluripotent cells with the ability of an unlimited, undifferentiated proliferation capacity *in vitro* and that can give rise to tissues of all three germ layers [36]. These characteristics render them favorable tools for basic research studies as well as translational research, in particular for studies on genetic diseases. However, human ESCs are gained solely by removing the inner cell mass (ICM) or parts of it from blastocysts which have been gained through *in vitro*-fertilization [37–39]. These methods have raised major ethical concerns, which lead to either restricted usage under stringent guidelines or prohibition of using hESCs in biomedical research.

Since the groundbreaking achievement of somatic reprogramming, hiPSCs have been regarded as ethically acceptable alternative to hESCs. HiPSCs are generated from already differentiated adults cells such as fibroblasts through somatic reprogramming by four factors – namely Oct3/4, SOX2, Klf-4 and c-myc [40]. These cells have the capacity of an unrestricted self-renewal and can give rise to all three germ layers, thereby combining its unlimited availability with its potential to differentiate in nearly every cell type. Further, hiPSCs offer promising options for disease modeling *in vitro* as well as a patient-specific drug testing in personalized medicine.

2.3. Differentiation of iPSCs into hepatocytes

HLC generation is highly dependent on the efficiency of endodermal commitment, hepatoblast formation, hepatocyte differentiation and hepatocyte maturation [41, 42]. Within these processes, two steps are of major importance: successful and proper definitive endoderm (DE) induction and final hepatocyte maturation. Although proof of lineage commitment is important to generate homogenous cell populations to avoid bias due to use of cells with inconsistent fetal cellular stages [43], many studies neglected to proof DE cell commitment [42]. Characterization of HLCs for proper expression of maturation markers *i.e.* morphology, binucleation, CYP expression, aspartate aminotransferase and alanine aminotransferase levels, urea and albumin secretion, glycogen storage capacity and increase in mitochondrial mass is thus imperative for standardized diseases modeling, drug screening and exploration of disease-related and patient-specific genetic backgrounds [44]. Various differentiation strategies were implemented for HLC generation using simple as well as complex multistep protocols on colony type, non-colony type (single cell approaches) and embryoid body (EB) formation. The following section should give some insight into the complex landscape of different HLC differentiation strategies.

2.4. Induction of definitive endoderm: Fine-tuned recapitulation of early embryonal development in vitro

Differentiating hiPSCs towards the hepatic lineage is a prolonged process performed in multiple stages of cells derived from DE precursors [42, 45, 46]. In murine embryonal development, cells of the ICM give rise to extraembryonic primitive endoderm (PrE) leading to the formation of parietal (PE) and visceral endoderm (VE) [47, 48] as well as ICM cells contribute to embryonic epiblast formation. Within this early multicellular structure, epiblast cells finally contribute to embryonic DE after primitive streak formation followed by a bipotential mesendoderm stage [42, 46, 49] and epithelial-to-mesenchymal transition (EMT) [45]. For many years the existence of such a bipotential precursor of mesoderm and endoderm was regarded as a speculative concept [46] due to close developmental proximity within the primitive streak until recent research revealed partial temporal overlap in mesodermal brachyury (T) and definitive endodermal FOXA2+/SOX17+ expression [42, 45, 46, 49]. Further DE development could be restricted to mesodermal cells expressing T (T+ cells) [45, 46] whereas cells that do not express T (T- cells) follow ectodermal fate [46]. The recapitulation of embryonal developmental stages with the induction of DE formation as a major critical step in HLC differentiation represents an important factor that has to be carefully monitored for a reliable and homogenous cell differentiation. A proper discrimination between the different endodermal lineages is necessary, since these cell stages share common markers expressed during the initial induction process [45], a problem often neglected in HLC generation [42].

2.5. Definitive endoderm formation in vivo is guided by Activin/Nodal, Wnt, BMP and FGF signaling pathways

During early embryonal development the formation of the primitive streak at the epiblast results in defined structures within three principle germ layers. The DE is formed by migration of precursor cells through the primitive streak that displace the VE and thereby distribute in an anterior-posterior fashion. Subsequently, adjacent germ layers contribute to endodermal anterior-posterior patterning and specify cell fate. The early anterior VE is known to secrete Nodal to these structures which relay it back to the anterior of DE. Finally, this cell sheet forms the three-dimensional gut tube with anterior DE contributing to ventral gut tube (foregut) which later gives rise to the liver bud [41]. Nodal and associated Wnt signaling were shown to be crucial for an efficient hiPSC-derived DE formation *in vitro* [44, 50–52]. In addition, after final gut tube formation, foregut tissue is adjacent to cardiac mesoderm and septum transversum which secrete FGFs and BMPs towards the foregut [44]. Both pathways are also involved in DE patterning *in vitro* [44, 50, 53].

In human iPSC-derived DE formation Nodal, Wnt, BMP-4 (members of the TGFβ superfamily) and FGF signaling specify cell fate (Fig. 1). Nodal and BMPs bind to type II receptors like Activin receptor type II

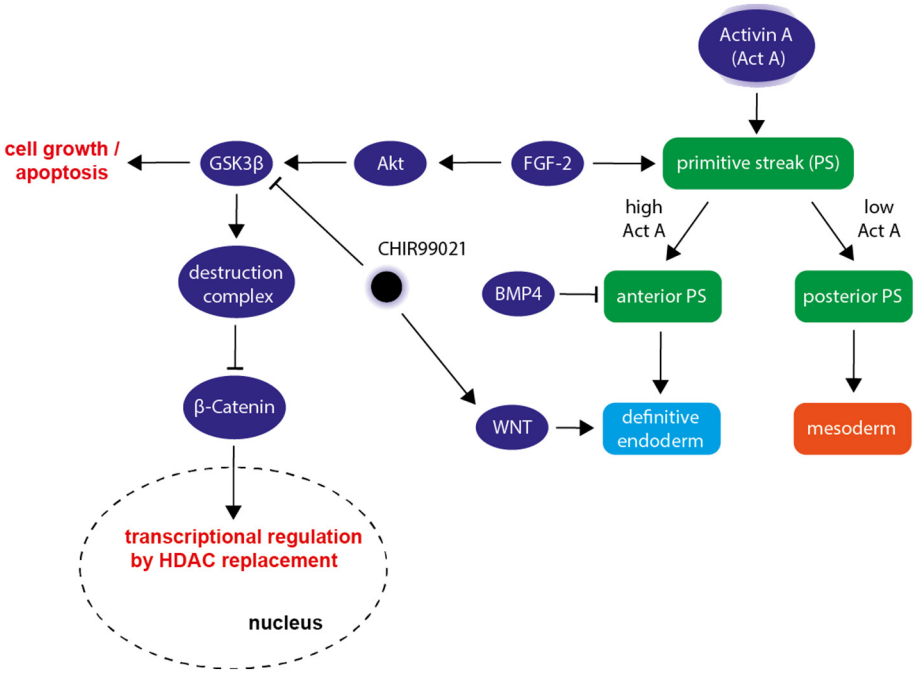

Fig. 1. Signaling pathways involved in DE induction. ActivinA/Nodal and Wnt stimulation trigger a complex and well-orchestrated regulatory network required for DE induction. High doses of Act A supported by FGF-2 favors DE induction whereas low Act A concentrations yield in mesodermal cells. Wnt supports DE induction by preventing β-Catenin degradation which enables β-Catenin-depended transcriptional regulation. The small molecule CHIR99021 is a potent inhibitor of GSK3β and even more effective in Wnt signaling induction than the natural Wnt ligand Wnt3A.

(ActRII A/B) and type I receptors (ALK4 or ALK2/3/6, respectively) [44] inducing Smad signaling events and complex formation [44, 54]. Smad complexes subsequently translocate to the nucleus [44] and induce DE formation by transcription of the genes FOXA2 and SOX17 [50, 51]. The canonical Wnt signaling pathway has been found to fulfill two important purposes supporting Nodal signaling. First, Wnt signaling eventually leads to GSK3β inhibition which has been shown to improve DE formation [51, 55]. GSK3β inhibition prevents downstream apoptosis in hiPSCs and contributes to their proliferation [56]. Second, canonical Wnt signaling preserves β-Catenin integrity by inhibiting the assembly of the destruction complex. Subsequently, β-Catenin translocates into the nucleus and displaces histone deacetylases (HDAC) that act as transcriptional repressors [44]. BMP-4 signaling is further closely associated to FGF signaling in hiPSC-derived DE. BMP-4 has been shown to modulate mesodermal fate towards ectodermal [45, 57] and endodermal structures since BMP-4 treated cells were unable to express T [50]. However, BMP-4 signaling in interaction with FGF-2 signaling contributes to differentiation towards mesendoderm [50] as BMP-4 blockage in presence of FGF-2 induces the expression of DE markers FOXA2 and SOX17 [58]. Similarly, in combination with Nodal signaling, FOXA2 and SOX17 expression [53] is also induced by BMP-4, that is integrated in a signaling network with strict control of the complex formation. FGF signaling acts also synergistic to Nodal but requires upstream TGFβ signaling as it has been shown that FGF alone cannot induce DE formation and is thus regarded as a supporting factor [57]. FGF signaling also acts directly on PI3K/AKT signaling, a pathway shown to be important for proliferation of iPSCs [49, 59]. Suppression of PI3K signaling by low inhibitor concentrations or a reduced stimulation by FGF or IGF induces mesendoderm and DE formation. However, complete inhibition of PI3K signaling results in high cell death rate through induction of apoptosis [49]. Sumi et al. already highlighted the complex signaling of Nodal, Wnt and BMPs and the need for their well-balanced interaction in hiPSC lineage differentiation (Fig. 1) [58]. However, more detailed research will be necessary to fully uncover the spatiotemporal regulation of inter- and counteraction of these proteins in mesendoderm and DE formation.

2.6. Strategies for efficient DE formation in vitro

Initially, Nodal signaling was found to be a major inducer in DE formation. Consequently, early protocols focused on stimulating this pathway. Activin A (ActA), another TGFβ family member, binds receptors specific for Nodal, except its co-receptor cripto [45], and showed sufficient efficiency in early studies in *Xenopus* to induce endoderm [60, 61]. This characteristic has been used, in presence of very low serum concentrations, as a first strategy to guide hESC towards DE formation [45, 46, 62]. High ActA concentrations of 100 ng/ml specify endodermal cell fate whereas low ActA concentrations favor mesodermal cell fate (Fig. 1) [46]. This protocol was quickly adapted as standard procedure for DE formation but is also associated with accumulating numbers of dead cells, especially in murine cultures [42, 63]. This observation might be related to undefined serum factor concentrations *i.e.* high FGF or IGF levels triggering augmented PI3K suppression [49]. Subsequently, strategies directing higher DE formation efficiency have been elucidated. Activation of Wnt pathway *via* Wnt3a ligand parallel to ActA signaling increased mesendodermal cell fate and induced subsequent DE commitment through inhibition of GSK3β that is associated with a higher rate of viable cells [44, 56, 62]. CHIR99021, a selective GSK3β inhibitor, has been shown to favor DE lineage more efficiently than ActA/Wnt3a when applied at low concentrations of 3 μM [51] or 5 μM within a short treatment regime of one day [55]. Interestingly, seeding densities have a profound impact on final DE induction efficiency. Initial low seeding densities of 2×10^4 cm^{-2} (approx. 10% starting confluency) specified hiPS cell fate with highest efficiency towards DE whereas of 8×10^4 cm^{-2} (approx. 30% starting confluency) showed a dramatic reduction in FOXA2 and SOX17 expression [51]. Further, in most studies differentiation was initiated on colony-type cultured cells which restricts the efficiency of how molecular triggers are able to access the cells. It has been proposed to use non-colony type culture strategies for more efficient differentiation [64]. This may explain some contradictory or divergent results found in the literature and points towards comparative studies to establish a single standard protocol for DE induction in hiPSC cultures.

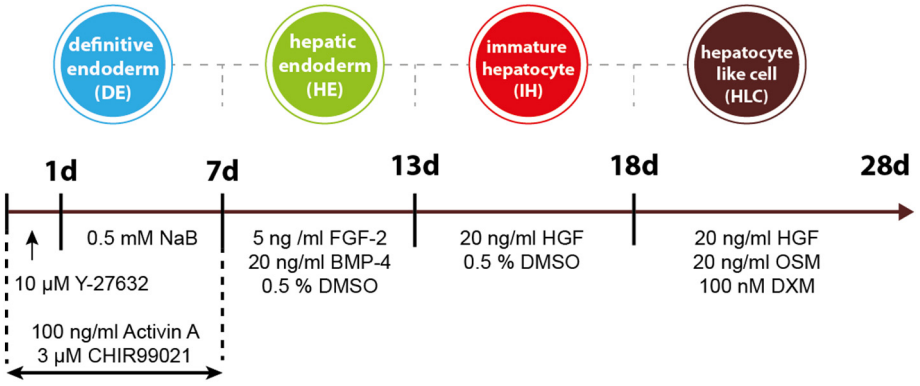

Fig. 2. HLC Differentiation protocols. Differentiation of iPSCs to HLCs involves four differentiation steps guided by several regulatory factors. The protocol is based on methods developed by Kajiwara et al. [76] and Peters et al. [77]. Seeding of single cell suspensions treated with Y-27632 at lower cell densities promotes Act A depended DE induction. Subsequently FGF-2 and BMP-4 shift cells in the DE stage towards hepatoblast formation. Hepatocyte differentiation is induced by the application of HGF. Immature hepatocytes maturate to iPSC-derived HLCs in media supporting hepatocyte culture supplemented with HGF, Oncostatin M (OSM) and Dexamethasone (DXM).

Single cell culture of hiPSCs is often associated with increased cell death. *E*-Cadherin mediated cell-cell contacts are necessary in hiPSC culture to prevent Rho-associated kinase (ROCK) triggered myosin hyperactivation resulting in membrane blebbing and apoptosis [65]. Further, Wnt induces ROCK activity *via* the non-canonical planar cell polarity pathway [66] and could trigger increased cell death even in colony-type culture, *i.e.* in response to Wnt3a or CHIR99021 treatment. Watanabe et al. recently showed in hESC promising results for Y27632 treatment, is a highly potent ROCK-I inhibitor [67], that prevented cell death and maintained cell pluripotency in long-term culture with a treatment concentration of 10 μM [68]. Other studies confirmed these results with various treatment times of Y27632 prior to DE induction and demonstrated a significant increase in gene expression of T, MIXL1, FOXA2 and SOX17 [69]. Y27632 can thus not only preserve cell viability but also prime hiPSCs towards mesendoderm and EMT stage promoting DE lineage commitment. Additional efforts have been made to streamline costs in differentiation associated with ActA guided DE induction, as peptide production/ purification is cost and time consuming. The small molecules inducer of definitive endoderm (IDE) 1 and IDE2 have been established [54] that act as TGFβ analogs and induce Nodal/ActA signaling [50]. However, DE induction by IDE1/2 stimulation falls below the efficiency observed for ActA treatment [54].

Nevertheless, small molecules have further been useful to guide mESCs towards skeletal muscle *via* Wnt activation and sonic hedgehog inhibition, where so far, no comparable strategy existed [70]. Additionally, sodium butyrate (NaB) is a thriving candidate of small molecules supporting *in vitro* hepatocyte differentiation. Early studies suggested a beneficial effect on maintaining the differentiated state of mature, primary hepatocytes and on the attenuation to acquire fetal characteristics, signs of dedifferentiation [71]. Moreover, NaB has been reported to support hepatocyte differentiation from mESCs [72, 73] and contributes to pure hepatocyte enrichment of up to 70% in presence of ActA [42]. NaB acts as HDAC inhibitor and is associated with cell growth arrest facilitating differentiation, contributing to hepatic phenotype by regulation of different HNFs, induction of CYP expression and stimulation of liver-specific genes [74]. Chemically synthesized compounds thus have the potential for a direct manipulation of signaling targets to determine the cell fate of choice [75].

Based on the available strategies, we propose DE induction through high dose ActA treatment (100 ng/ml) supported by 3 μM CHIR99021-mediated GSK3β inhibition, low millimolar NaB supplementation and initial short-term (24 h) ROCK inhibition through 10 μM Y27632 application in RPMI medium with xeno-free B27 supplementation (Fig. 2). Moreover, these defined, serum free conditions help to reduce bias of undefined, serum-derived compounds [51] and facilitate the translation of HLC into appropriate disease and drug testing models.

2.7. Strategies for hiPSC purification and maturation to HLC

HLC maturation and its purification from remaining fetal cell populations are of major importance for subsequent applications in *in vitro* models. The 3D microenvironment of the target tissue could also support final maturation *in vitro*. To date, past DE induction most sophisticated protocols follow the same hepatic specification and maturation strategies applying FGF-2 and BMP-4 [76–78] or DMSO, Oncostatin M (OSM) and hepatocyte growth factor (HGF) [42, 63, 76–80], respectively, as they are required for hepatocyte differentiation from endodermal cells [81]. Some protocols rely on the application of serum with unspecified factor composition during hepatic specification phase [42, 76], most likely fulfilling FGF-2 and BMP-4 signaling. However, use of undefined serum with varying composition that relies on its source and the provider could potentially contribute to bias in cell differentiation and might restrict the reliability of already established protocols. During early liver development, FGF signaling is crucial and secreted by the cardiac mesoderm [44]. It has been shown that FGF signaling is induced in cardiac mesoderm precisely by the time of hepatogenesis from ventral foregut endoderm. FGF-2 signaling induces serum albumin mRNA and AFP mRNA, early hepatic lineage markers of hepatoblast stage, within sharp concentration thresholds and in a tissue-specific manner [82]. Likewise, BMPs are secreted from the septum transversum during liver bud formation [44, 83]. BMP-4 could successfully initiate hepatic specification from mESC-derived DE cells [83]. Further, BMP-4 primes cell fate decision and renders the ventral foregut endoderm susceptible to FGF-2 *via* GATA-4 transcription factor increase. This concerted program is required to also downregulate pancreatic gene expression which is assumed to be the default developmental program of ventral foregut endoderm [84, 85]. Liver maturation has been shown to be dependent on OSM and HGF availability. Both factors are able to induce albumin production and glycogen accumulation within fetal hepatic cells. OSM induces late fetal liver development, whereas HGF predominantly occurs in neonatal liver and induces post-natal liver maturation [86]. Nevertheless, many studies show less mature hepatocytes and HLC remaining at fetal stages with low expression of maturation markers, protein secretion levels and metabolite formation [42, 43, 77, 78]. Expression of fetal markers and their decline is often infrequently thoroughly addressed. In the course of HLC differentiation, fetal AFP expression should peak during hepatoblast stage followed by a significant decline when hepatocytes become mature [42]. In addition, the decline of CYP3A7 expression in early hepatoblast cells represents another valuable checkpoint for HLC maturation [80]. Kajiwara et al. recently highlighted donor-dependent variations in hepatic differentiation potential of hiPSCs [76]. These variations likely contribute to frequently observed varying efficiencies in the cell differentiation process. Thus,

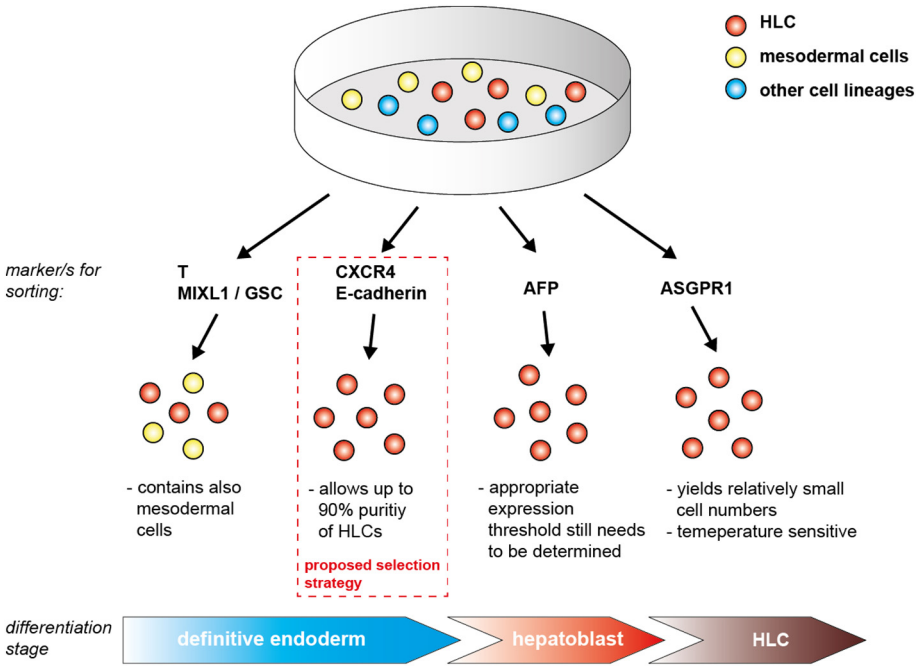

Fig. 3. HLC differentiation markers and purification strategies. iPSC-derived HLC differentiation results in mixed populations with different cell differentiation stages. Various enrichment strategies were proposed to allow purification of differentiated HLCs. Early DE stage enrichment via mesendodermal T expression combined with MIXL1 and GSC may still yield in mixed populations with mesodermal specifications [88]. CXCR4 positive selection in combination with E-Cadherin expression at final DE induction was shown to generate populations with 90% HLCs and may represent the most efficient method to produce large HLC populations (framed with dashed lines) [63, 87]. Subsequent hepatoblast stage expresses AFP at high levels representing another marker for HLC enrichment. However, defined AFP expression thresholds have to be established to exclude undifferentiated cells and cells of other lineages since AFP is expressed in all iPSC differentiation stages of the hepatocyte lineage [42, 78]. ASGPR1 enrichment during HLC differentiation is discussed to yield in highly specified hepatocytes. Yet, ASGPR1 expression is reported to be sensitive to changes of the surrounding temperature and the marker is expressed only in small cell populations (<30% of differentiated cells) [79, 89].

purification of cell populations obtained during differentiation should be performed.

Several markers have emerged as appropriate candidates for HLC enrichment in the process of its differentiation and maturation. Kubo et al. reported that upon the initiation of hepatic differentiation only T + cells commit to DE (expression of FOXA2 and SOX17) and subsequently contribute to hepatic lineage (further albumin expression). This was already observed after a few days of differentiation [46]. Thus, T represents a suitable early selection marker to enrich cell populations (Fig. 3). However, complementary markers should be analyzed in addition to T at later HLC differentiation stages, as T expression is transient during mesendodermal stage [51] and it is further described also as a panmesodermal marker [87]. Nevertheless, its expression together with other mesoderm markers like MIXL1 or GSC prior or parallel to upcoming endodermal markers should be closely monitored to guarantee a guided differentiation through mesendodermal stage and to distinguish mesodermal T(+) cells from endodermal T(+) cells [88]. After mesendoderm and DE induction hepatic lineage commitment is detectable in the hepatoblast cell. At this stage alpha-1-fetoprotein (AFP) can be used as a reliable marker that is increasingly expressed at hepatoblast stage, but expressed at low levels in undifferentiated hiPSCs and mature liver cells [42, 78]. Although the analysis of additional hepatoblast markers such as CYP3A7 [42, 80] and FOXA2 [78] has been suggested, monitoring of AFP expression offers the advantage of being continuously detectable during the whole HLC differentiation process. For an enrichment of HLC at later differentiation stages detection of ASGPR1 expression has been proposed [77, 79]. Cells sorted by ASGPR1 selection showed gene expression profiles similar to PHH, but only 26% of finally differentiated cells were found positive for ASGPR1 expression [79]. Further, analysis of this protein should be performed with caution, as its surface expression has been reported to be temperature-dependent and to change within minutes [89]. Most promising results for HLC enrichment have been shown by selection of CXCR4+ cells at early DE stage [45, 49, 55, 63, 87, 90]. CXCR4 is expressed in developing DE and mesoderm, but not in VE or PrE [91] and has been also used for cell selection in mESCs and hESCs [45, 87]. Several *in vitro* studies showed high correlation of CXCR4 and DE marker expression in up to 90% of cells of the DE population [45, 49, 90]. Cells selected for CXCR4 expression have been shown to successfully differentiate very efficiently to the hepatic lineage [87]. Notably, two studies reported complementary selection for *E*-Cadherin expression to be beneficial for a stringent DE selection [63, 87].

2.8. Novel hiPSC-derived hepatocyte maturation strategies: Recreating the in vivo microenvironment

In most of the studies published so far, HLC differentiation remained at fetal stages due to still insufficient differentiation strategies. The *in vivo* microenvironment of differentiating stem cells is comprising numerous soluble factors for guidance of embryogenesis and early differentiation. In contrast, reductionistic approaches of cell differentiation *in vitro* currently rely on the stimulation with isolated growth factors under artificial cell culture conditions. It is very likely, also other parameters of the microenvironment, *i.e.* substrate elasticity or mechanical forces will influence cell differentiation [92]. In accordance with this assumption, undifferentiated hiPSCs have been shown to respond to mechanical stimulation with cytoskeleton rearrangements, expression of cell adhesion molecules and activation of specific signaling pathways [93]. Substrate stiffness, 3D matrices, cell-cell interactions, mechanical strain and shear stress might support maintenance of pluripotency and contribute to cell differentiation in a spatio-temporal manner. However, the underlying mechanisms for endodermal cells such as the hepatic lineage still need to be elucidated, as most of the research conducted so far has been done with focus on ectoderm and mesoderm differentiation [92, 94].

2.8.1. Cellular cross-talk mediates hepatocyte maturation

Cellular cross-talk plays an important role in regulating cellular differentiation and adaption to the microenvironment. NPC have been shown to stabilize hepatocyte differentiation by improving their metabolic function and maintaining the hepatocyte phenotype [95–97]. A recent study demonstrated that HSC form a liver-resident mesenchymal stem cell population that contributes to liver regeneration in by forming mesenchymal tissue, progenitor cells, hepatocytes, and cholangiocytes [98]. The importance of NPC co-culture for preservation of hepatocyte phenotype and function was further recently proofed *in vitro* by Esch et al. demonstrating improved albumin and urea secretion by PHH co-cultures with fibroblasts, HSC and KCs [99]. Hence, advanced co-culture approaches emerge a favorable strategy to also improve hiPSC-derived hepatocyte differentiation. In this context immobilized signaling molecules such as HGF, bFGF and BMP4 involved in HLC differentiation showed superior improvement of DE-commitment compared to soluble factors [100]. Cell substrates of HGF mixed in a matrix with fibronectin and collagen as well as ECM proteins derived from co-culture of HLC with mESC colonies were found to improve HLC differentiation [100]. Furthermore, hepatoblasts co-cultured with hiPSC-derived LSEC have been shown to mediate an increase of hepatic enzyme expression compared to HLC mono cell cultures [101]. In this study, the combination of HGF, FGFs and BMPs secreted through LSEC with the use of ECM-matrix proteins as cell substrate was shown to be a successful approach to further improve HLC differentiation and its function. Interestingly, also mesenchymal stem cells (MSCs) show a comparable potential for supporting hepatocyte differentiation. Tagaki et al. showed positive effects of MSC co-culture on hiPSC-derived hepatoblast maturation with an increased protein secretion rate and improved CYP3A4 enzyme activity [102]. Kadota et al. generated a whole-liver graft with enhanced hepatic functions of primary hepatocytes in co-culture with MSCs and successfully implanted it into rats [103]. MSCs improved tight hepatocyte parenchyma formation by the secretion of laminin and other ECM-derived proteins. These studies highlight the importance of cell-cell communication, endogenous secretion of growth factors and composition of the cell substrate for hepatocyte maturation *in vitro*.

2.8.2. ECM-derived factors further support hepatocyte maturation in 3D arrangements

The interaction of cells with the surrounding ECM is an important regulator of cellular adhesion, proliferation, migration and differentiation. Primary hepatocyte function was found to be remarkably improved by 3D co-culture strategies [104]. Nanofibers made of gelatin and poly lactic acid (PLA) that were coated with Matrigel© improved DE induction in hiPSC-based cultures by facilitating the cellular growth in a 3D arrangement [54]. However, it was also shown that nanofibrous PLA scaffolds are less effective in preserving albumin secretion and CYP activities in HLC compared to *ex vivo* ECM scaffolds derived from decellularized liver tissue [105]. Human HLC maintained in scaffold-free embryonic bodies (EB) have also been reported to improve the differentiation and maturation potential associated with increased CYP expression levels compared to 2D monolayer cultures [106, 107]. However, EB-based differentiation resulted in heterogeneous cell populations due to gradient formation of nutritional components and did not reach metabolic capacity of PHH [106].

Hepatocyte culture might be further supported by co-culture with HSC that provide an actively secreted ECM scaffold. Micropatterning of different cell types represents a promising approach to control cell-cell and cell-matrix interactions of HLCs with NPCs in defined arrangements. Micropatterned co-cultures of HLCs with swiss 3T3-J2 murine embryonic fibroblasts in an ECM sandwich scaffold have been shown to significantly improve hepatocyte maturation and to stabilize its phenotype [108]. The precise arrangement with 3T3-J2 cells in contact with HLCs resulted in increased expression levels of various genes involved in drug metabolism, improved metabolic activity and the elevated synthesis of albumin for several weeks [108]. Swiss 3T3 murine embryonic fibroblasts secrete considerable amounts of collagen-I supporting hepatocyte maturation. Nagamoto et al. overlaid HLC cultures after differentiation with 3T3 fibroblast cell sheets to improve HLC gene expression of various proteins, *i.e.* CYP enzymes [104]. In an approach termed RAFT (real architecture for 3D tissue) Gieseck et al. arranged HLC in 3D collagen constructs, which increased albumin secretion and CYP3A4 activity for several weeks compared to 2D cell culture [109]. Furthermore, canalicular structures, cell polarity and cell-cell contacts longevity of HLC were preserved by this cell culture technique. Another interesting approach combines NPC co-culture of HLC with ECM scaffolds arranged in a bioinspired setting. The microanatomical structures were recreated by bioprinting of the ECM matrix to enable the development of a 3D biomimetic liver model that recapitulates the liver module architecture and supports hepatocyte maturation [110].

2.9. hiPSC-derived LSEC and immune cells

Most of the publications in the field of generating hiPSC-derived liver cells focused almost exclusively on hepatocytes. Although hepatocytes are the predominant cell type of the liver, NPC including LSECs, KC and HSC are essential to maintain hepatic metabolic activities in addition to handling other functions that are not covered by hepatocytes [111]. Thus, generation of these cell types from hiPSCs would offer the option of generating a genetically matched liver models comprising HLCs as well as NPCs. However, only a few studies are available that describe the generation of hiPSC-derived NPCs. Du et al. obtained hepatocytes and endothelial cells in parallel by differentiating hiPSCs, resulting in a construct which contained genetically identical endothelial cells and HLC. Endothelial cells co-cultured on a hydrogel scaffold with HLC significantly improved hepatocyte function and facilitated vascularization of the scaffold when implanted in a mouse partial hepatectomy model [112]. Recently, Koui et al. published a study in which the authors demonstrated the differentiation of LSEC and HSC from hiPSCs. In the co-culture, both cell types promoted self-renewal of hiPSC-derived liver progenitor cells over the long term in a two-dimensional culture system without addition of exogenous cytokines and hepatic maturation factors [101].

In addition to NPC, also lymphocytes and myeloid cells of the blood are central players of the immune response at the liver sinusoid. A significant progress has also been made towards generating functional immune cells from hiPSC lines. Generation of functional DC-like cells has been first described by Zhan et al. [113]. Functional DC-like cell with the ability of phagocytosis and antigen presentation ability to T cells have also generated by others [114]. Recently also T cells have been generated from hiPSCs [115–117] as well as functional NK cells [118–120] that express NK cell-associated inhibitory and activating receptors, exhibit cytolytic function and cytokine production [121], and that exhibit *in-vivo* anti-tumor response [122]. Further, the generation of macrophages from hiPSCs has been shown by a couple of groups [123–125]. In one of these studies, Yeung et al. described an approach using macrophages derived from hiPSCs (iPSdMph) to study macrophage–Chlamydia interactions *in vitro*. The authors could demonstrate, that iPSdMph are able to support the full infectious life cycle of *C. trachomatis* in a manner that mimics the infection of human blood-derived macrophages [126]. In addition, also granulocytes have been differentiated from hiPSCs and shown to suitable for disease-modeling studies [127]. These studies demonstrate that besides the generation of HLC, also other cell of the human liver can be reliable differentiated from hiPSC lines. This exiting work paves the way for new options in personalized medicine as novel tools for disease-modeling and drug screening by generation of liver tissues incorporating all major cell types of the liver derived from one patient-specific genetic background.

3. Perfusion systems for emulation of the human liver *in vitro*

3.1. Scaffolds supporting cell growth and differentiation

Soft lithography and replica molding using polydimethylsiloxane (PDMS) are often used as microfabrication techniques for manufacturing biochips that allow a precise control of cell growth and intercellular interaction, fluid flow rates and compound exposures. PDMS is widely available through commercial sources and allows the generation of solid chip devices with transparent properties that allow for on-chip imaging of integrated cell layers [128]. PDMS has quickly become the most prevalent substrate for biological microfabricated devices as a result of its low cost, low cytotoxicity, and ease of processing ([129]). Despite the many favorable properties, PDMS has significant limitations. Most importantly, due to the hydrophobic nature of the material small hydrophobic molecules are efficiently adsorbed at the surface [130]. Further issues occur when considering the material permeability, as PDMS allows exchange of gas and water evaporation which limits its ability to maintain constant environmental conditions within cell culture devices [128]. Further, remaining non-crosslinked PDMS monomers have been shown to negatively affect cellular behavior [131]. Due to the aforementioned reasons, other biomaterials already established in cell culture for decades, such as polystyrene or polycarbonate are incorporated into MPS platforms. 3D bioprinting methods allow for inclusion of suitable cell culture scaffolds that provide structural support and integrity for the developing liver tissue *in vitro*. The substrate must possess the adequate mechanical strength to support the cells but also provide appropriate porosity to allow growth factor diffusion and cell growth. Most important, the scaffold needs to be biocompatible with no toxic effects to the cells. Ideally, the substrate does not only provide biochemical but also mechanical stimulation to influence cellular differentiation and function. Among various mechanical factors, matrix elasticity has a crucial role in the induction of cellular responses and cell fate including proliferation, differentiation, migration, adhesion, and maturation [132]. Mechanical properties of the scaffold further determine the capacity of the tissue to resist to shear force during perfusion. Matrices such as hydrogels aim to recapitulate the composition, structure, and function of the extracellular matrix (ECM) that normally surrounds liver cells. These hydrogels offer the possibility to enrich the cell substrate with growth factors and other supplements required to regulate cell differentiation and maturation. Matrices of recombinant ECM proteins have been shown to improve hepatocyte phenotype and maturation in 2D and 3D platforms [111]. Current biomaterials have proven to be cost-effective and highly reproducible to significantly reduce batch variation. Cell encapsulation by hydrogels can be used for controlling the maturation, size and microenvironment of developing tissue [133]. Natural materials such as laminins or alginate [134, 135] as well as synthetic materials *i.e.* polyvinyl alcohol (PVA), polylactide-co-glycolide (PLG), poly [2-(methacryloyloxy) ethyl dimethyl-(3-sulfopropyl) ammonium hydroxide] (PMEDSAH) or poly (caprolactone) (PCL) have also been used as cell substrates with good results in supporting maintenance of the cell phenotype [136, 137].

3.2. Microphysiological models of the human liver

Microphysiological systems (MPS) of the liver (often termed as liver-on-chip) aim at recapitulating a microphysiological liver environment by 3D cell culture. MPS represent valuable tools for the identification of molecular targets and the testing of tailored treatment strategies under physiological conditions *in vitro*. Several MPS emulating the liver function have been described in the recent years. However, most of these models were intended to be used for studies on adsorption, distribution, metabolism, elimination and toxicity (ADMET), and pharmacokinetics studies of drugs. Typically, microfluidic perfused biochips made of biocompatible polymeric and transparent material serve as cell culture devices for hepatocytes that are often co-cultured with NPC. These devices enable physiological growth and tissue differentiation by regulation of cellular positioning, *i.e.* through cell patterning and 3D bioprinting [138–140], regulation of shear stress and related mechanical forces [2, 141–144]. They further act as scaffold to provide cell–cell and cell–matrix interactions in a biomimetic microenvironment [145, 146]. In the recent years microfluidics and biochip technology have overcome many limitations of traditional static cell culture platforms [147]. The technology provides a precise spatiotemporal control of growth and differentiation niches through accurate delivery of exogenous factors such as oxygen or nutrition. Perfusion of the biochips also provides an efficient method for removal of cellular waste products that enables a sustained tissue homeostasis. Biochip platforms for MPS culture typically allow imaging of cells with various microscopy techniques and could integrate advanced sensors for continuous monitoring of central cell culture parameters such as cell viability (*e.g.*, transepithelial/transendothelial electrical resistance (TEER)), oxygen and pH, and even allow in-line analyses of chemokines and metabolite formation [148–151]. A continuous monitoring and adjustment of these parameters provides the basis to maintain a biomimetic niche that supports growth and differentiation of hiPSCs. It was demonstrated that perfusion of premature HLC in microfluidic biochips triggers upregulation of several pathways related to cellular reorganization, stress response and drug metabolism. However, a direct perfusion of HLC causes the upregulation of genes related to endothelial biliary cells differentiation and resulted in dedifferentiation to a fibroblast-like phenotype [152] (Fig. 4).

In vivo, hepatocytes are not subject to direct flow as they are protected from shear stress by the sinusoidal endothelial lining and the space of Disse. This might explain the fact that also HLCs are highly sensitive to direct shear stress and react with a decreased growth rate and increased cell death numbers at high perfusion flow rates [153]. The integration of a physiological barrier resembling the function of the endothelial lining would thus be a more a suitable perfusion setting [149]. A few systems integrating such a bioinspired barrier were described for perfusion of hESC- or hiPSC-derived HLCs [106, 154–156].

Zeilinger et al. used a polyurethane housing containing three bundles of interwoven hollow fibers serving as cell substrates that enable the microfiltration and supply of nutrition and oxygenation. The bioreactor design was re-scaled from a large 800 ml bioreactor design initially intended for use in pilot studies to support liver function in patients to a 2 ml bioreactor for *in vitro* studies with a good performance in maintaining PHH function for up to three weeks [157]. This bioreactor system was recently also used to differentiate and maturate hiPSCs to HLCs [106, 155, 156]. The differentiation protocols from Miki et al. [155] and Sivertsson et al. [154] have been used to differentiate non-differentiated cells, DE-derived cells, cells from hepatic progenitors as wells as hepatoblast cells to HLCs. These studies allowed an interesting insight into 3D perfusion supported differentiation and demonstrated that the stage of the starting cell material did not greatly influence the outcome of differentiation. Moreover, it was demonstrated that indirect perfusion strategies of 3D cell cultures enable a superior nutrient and gas exchange which allowed for an improved HLC differentiation compared to conventional 2D strategies. HLCs in a perfused 3D microenvironment showed increased steroid hormone synthesis, xenobiotic and lipoprotein metabolism reflecting main functions of mature liver tissue. In addition, enhanced CYP expression, albumin and urea synthesis by HLCs has been demonstrated [155]. However, in the bioreactor model hESC-derived hepatocytes contained also fractions of cells with heterogonous differentiation stages. Only 30% of HLC expressed ASGPR1 and cells further showed less pronounced expression pattern of CYP enzymes compared to PHH [154] with some cell fractions still expressing fetal CYP3A7 [155]. Enhanced expression of HNF4a and CYP was also shown by Meier et al. in HLCs cultured in the 3D bioreactor [106]. Further, in the study by Freyer et al. the formation of bile duct like structures and improved albumin and urea production has been reported in 3D perfusion systems [156].

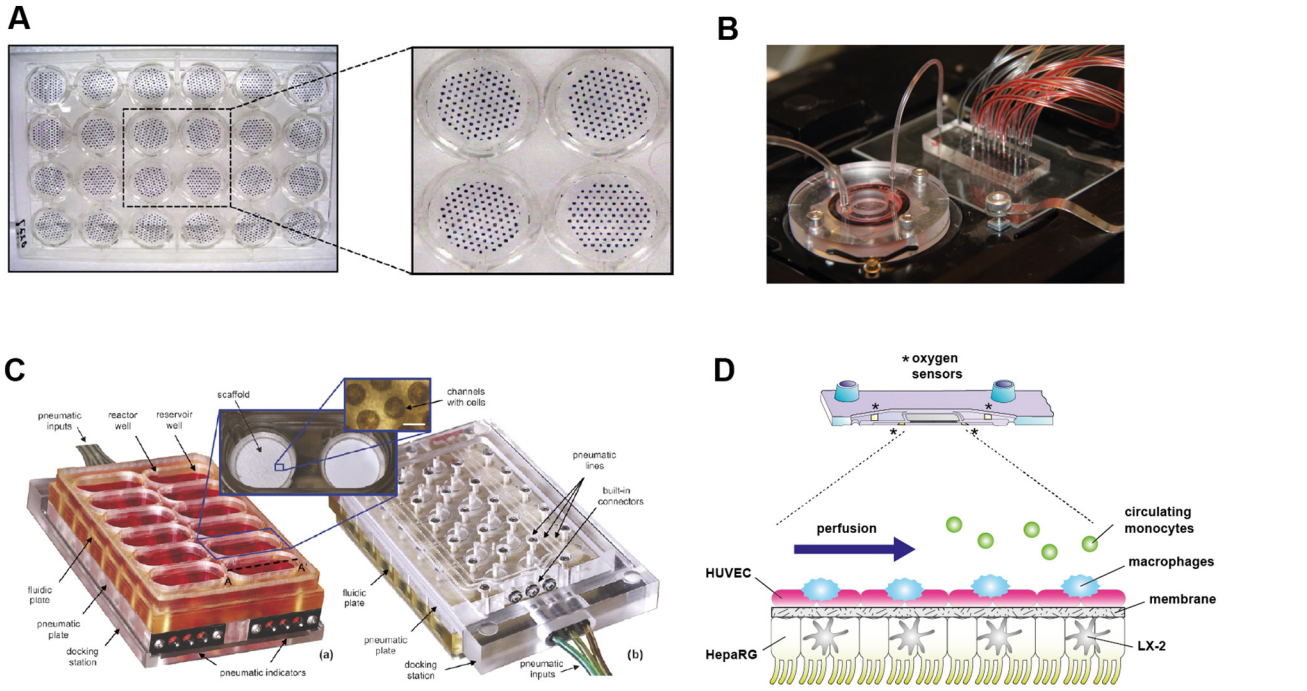

Fig. 4. Emulation of liver function in vitro. A) HLC co-cultured with 3 T3-J2 fibroblastes co-cultured as MPCC in a 24-well standard tissue culture polystyrene plate [108]. B) Setup of a miniaturized bioreactor connected to a computer controlled microfluidic switchboard. The system allows electrochemical measurements of glucose and lactate in metabolic studies of oxidative phosphorylation and anaerobic glycolysis in perfused hepatocyte cultures [176]. C) Perfused multiwell with an array of 12 liver-on-chip bioreactors. Multiwells contain built-in connectors and pneumatic lines distributing air pressure to individual valves and pump chambers for perfusion of hepatocytes [167]. D) Liver-on-chip model in MOTiF biochips. The liver model is composed of non-parenchymal cells (endothelial cells (HUVEC), macrophages, stellate cells) and hepatocytes. Cells are cultured in two layers separated by a membrane that are perfused with circulating immune cells through michrochannels resembling parts of the blood immune system [149, 182].

In the liver, hepatocytes are separated and protected by shear stress by the endothelial lining of the sinusoids. To recreate this endothelial–epithelial interface, microfluidic devices with microfabricated barriers were created that separate cultured hepatocytes from fluid flow [158]. Hepatocyte co-culture with additional cell types is a widely used approach to enhance hepatocyte activity. Bhatia and Khetani established micropatterned co-cultures (MPCC) by seeding hepatocytes and 3T3 fibroblasts on bioprinted collagen structures in 24-well plates [159]. In MPPC models hepatocytes were layered on a fibroblast feeder layer [160]. In both approaches hepatocyte function was found enhanced, including increased albumin synthesis and secretion, urea synthesis, and glycogen storage. Other studies reported MPCC platforms with improved hepatocyte function by co-culture with endothelial cells [112] and HSCs [161, 162]. MPCCs have also been applied for infection studies with several pathogens, such as hepatitis B virus ([163]), hepatitis C virus [164], and malaria [165].

The efficiency of MPCCs was further improved by perfusion in microfluidic devices.

Sivaraman et al. perfused hepatic aggregates adhering to the collagen-coated walls with an array of microchannels, resulting in higher hepatic functions compared to static conditions [166]. In a follow up study, the system was further improved by co-culture of rat hepatocytes with LSECs [167]. Co-cultures of hepatocytes with endothelial cells have also been established in other systems, however, due to their limited availability not always with LSECs. In a biochip bovine aortic endothelial cells were separated from hepatocytes with either an extracellular matrix (ECM) protein layer or a microporous membrane. The system allowed maintenance of hepatocyte function for up to 30 days [168] and was also used to analyze viral replication for hepatotropic hepatitis B virus [168]. Kasuya et al. induced the formation of capillary-like structures by bovine pulmonary microcapillary endothelial cells in a layered model with HSC and hepatocytes [169]. Another liver sinusoid model used two microfluidic chambers separated by a porous membrane that could be maintained for 28 days [170]. Weng et al. established a scaffold-free co-culture model of primary rat hepatocytes and HSC perfused with radial flow [171]. Enhanced hepatocyte function was also reported for primary rat hepatocytes co-cultured with 3T3-J2 fibroblasts under medium and oxygen perfusion [139]. A microfluidic platform was further used for culture of PHH monolayers [172], and co-cultures of PHHs and NPCs [173]. In addition, perfused co-culture systems have been established for spheroidal tissue models [174, 175].

For real-time measurement of metabolic changes biosensors have been integrated in biochips for MPS. We recently described the integration of luminescence-emitting sensor spots in biochips for real-time measurement of oxygen consumption by a multilayered co-culture model of endothelial cells, LX-2 stellate cells, primary macrophages and HepaRG hepatocytes [149]. Bavli et al. developed a biochip with a computer-controlled microfluidic switchboard that can simultaneously monitor mitochondrial respiration, glucose, and lactate in HepG2/C3A aggregates and that allows the monitoring of the shift from oxidative mitochondrial respiration to glycolysis in response to treatment with two hepatotoxins [176]. Even though hepatocytes in the liver are protected from flow induced shear stress by the endothelial lining, blood circulation within the sinusoid creates gradients of oxygen, nutrients, and hormones, which have been shown to lead to a phenomenon termed zonation that consists in the distribution of hepatocytes with *i.e.* defined CYP450 expression pattern across the length of the sinusoid. This differentiation pattern is induced and maintained by oxygen gradients [177]. The liver sinusoid is functionally divided into three zones based upon oxygen tension. The periportal zone (PPZ) rings the portal tracts, where the oxygenated blood from hepatic arteries enters and mixes in the sinusoid with blood from the portal vein. The perivenous zone (PVZ) is located around the central vein, where oxygen tension is much lower, and the intermediate zone (IMZ) is located in between PPZ and PVZ. Hepatocytes of the PPZ and PVZ can be distinguished based on its metabolic capacity, with increased CYP expression and drug metabolism in hepatocytes of the PVZ. Further, anaerobic glycolysis is performed in areas with limited oxygen availability,

predominantly in perivenous hepatocytes that express the key glycolytic enzyme glucokinase (GK). In contrast, gluconeogenesis by phosphoenolpyruvate carboxy-kinase 1 (PEPCK1) is preferentially carried out in the PPZ of the liver sinusoid. The expression levels of those enzymes further depend on the nutritional status and changes in hormone concentrations of insulin and glucagon [178, 179]. Enzymes involved in ureagenesis and beta oxidation of fatty acids are more active in hepatocytes of the PPZ, whereas enzymes involved in glutamine synthesis and lipogenesis show higher expression rates in hepatocytes located at the PVZ of the liver sinusoid [111]. To mimic zonation pattern *in vitro*, Allen et al. generated oxygen gradients in a bioreactor across rat hepatocyte/fibroblast co-cultures by cell-mediated depletion of oxygen dissolved in the culture medium [61]. Similar to *in vivo* conditions, the oxygen gradient led to higher expression of CYP450s in the hepatocytes subjected to the low-oxygen regions compared to the high-oxygen regions. A biochip providing a continuous oxygen gradient to cultured mouse hepatocytes was also described by Sato et al. [62]. Microenvironments of the PVZ and PPZ have also been modeled in a liver model comprising endothelial cells, LX-2 cells, PHH and monocyte-derived macrophages embedded in a microfluidic biochip device using computational modeling of oxygen tension [180]. Finally, Weng et al. demonstrate the creation of spatially-controlled metabolic zonation of rat hepatocyte cultures by concentration gradients of exogenous insulin and glucagon in a microfluidic device [181].

4. *In vitro* models of infection-related liver dysfunction

4.1. Inflammation-related liver dysfunction

In recent years our knowledge about the pathophysiological processes in the course of liver infections and its sequelae has largely expanded. However, the lack of suitable models able to reflect the complex communication processes within the human liver hampered the development of new drugs as elucidation of cellular and molecular host-pathogen interaction still need to be fully unraveled. We recently developed a microfluidic biochip, that allows the perfusion and long-term cultivation of bioengineered three-dimensional liver tissue [149]. The multilayered tissue comprises endothelial cells and monocytes-derived macrophages co-cultured in an endothelial cell layer that is separated by a scaffold membrane mimicking the space of Disse from hepatocytes and stellate cells forming the hepatic cell layer that is co-cultured opposite to the endothelial cell layer [149]. Monocytes were integrated into the endothelial perfusion circuit to emulate essential components of the innate immune system [182]. In this MPS we were able to resemble central aspects of inflammation-related liver dysfunction in response to PAMP stimulation. The immune response and the related cytokine profile was highly specific to the TLR agonist and resulted in typical disease-related alteration of hepatocellular function, such as impairment of MRP2 dependent bile secretion, decreased expression of tight and adherence junction proteins and depressed secretion of albumin and urea [182]. However, upon integration of circulating monocytes the activation pattern of tissue resident macrophages was rendered by invading monocytes that induced tissue remodeling with full recovery of metabolic function of the liver model after acute phase inflammation [182]. The liver-on-chip model is thus capable of mimicking disease processes as well as recovery from liver-dysfunction.

While true infections of the liver are relatively rare and most often are viral, development of liver dysfunction disrupts metabolic and immunological homeostasis in the critically ill patient with bacterial infection and frequently promotes progression to multiple organ failure. Accordingly, pre-existing liver disease is a risk factor for the progression of bacterial infections to sepsis with increased odds ratios for hospitalization, ICU admission, and death [183]. Similarly, patients with septic complications in the presence of chronic liver disease, most notably cirrhosis, have a poor prognosis due to development of acute-on-chronic liver failure (ACLF) [184].

The liver is highly sensitive to "septic" infections due to its central role in metabolism and detoxification. The excretory machinery of hepatocytes is particularly vulnerable to systemic inflammation and pneumonia reflects the second most frequent cause of jaundice in hospitalized patients only surpassed by malignancy of the head of pancreas [185]. Hepatocellular excretory failure secondary to sepsis is mediated predominantly through phosphoinositid-3-kinase (PI3K) [186]. Inhibition of this central signaling cascade in a host with life-threatening infection has significant limitations as recruitment of the 'first line of defense' against bacterial infection, *i.e.* granulocytes, explicitly depends on intact PI3Kγ mediated chemotaxis [187] (Fig. 5). As such, inhibition of PI3Kγ represents a promising strategy to prevent sepsis-associated hepatic dysfunction if off-target effects on the immune system can be avoided. An efficient and cell-specific delivery into hepatocytes (while deliberately avoiding uptake into immune cells) of inhibiting nanoparticle-based strategies underlines the potential of the organ-on-chip technology to evaluate such strategies. Screening of different carriers and functionalizing strategies depends on a microphysiological milieu in which the concurrent uptake can be studied between different cells under flow conditions [188–190]. Uptake characteristics of the nanostructures as well as their cellular effects can be investigated in MPS comprising target (hepatocytes) and off-target cell types (macrophages, endothelial and stellate cells) that are located in liver lobule in close vicinity.

4.2. Hepatic infection

Stem cell-derived hepatocytes have proved useful in delivering models for studying the lifecycle of hepatotropic viruses. Most frequent infection of the liver include viral infections with hepatitis viruses type A (HAV), B (HBV), C (HCV) and E (HEV); but also, bacterial and fungal infections as well as parasite infections with *Plasmodium* spp. causing malaria [163, 191–194]. The following section should provide a brief overview of viral and microbial infection diseases affecting liver function. These infections and the related alterations at the molecular and cellular level might be efficiently emulated in more detail in complex human cell culture models such as liver MPS to identify suitable targets for novel treatment strategies.

4.2.1. Viral infections

Hepatitis A virus (HAV) and hepatitis E virus (HEV) are food-borne pathogens that traverse the gut epithelial cell layer to reach the liver *via* the blood stream. Although HAV is able to replicate in different cell types *in vitro*, *in vivo* its replication is limited to the liver [195]. It has been proposed that HAV-specific IgA antibodies produced in the intestinal mucosa bind to circulating HAV and can serve as carriers of the virus to the liver. Fcα receptor expressed by KC is than binding to IgA–HAV complexes and supports transfer of the virus to hepatocytes. However, this infection model remains elusive and needs further investigation [196]. Infections with the hepatitis E virus (HEV) are particularly life threatening in pregnant women and may cause a chronic infection with increased risk of cirrhosis in immunocompromised individuals [197]. However, up to date no specific antiviral drug treatment is available. Recently infection and replication of primary isolates of HEV was investigated in HLCs derived from hESC. CRISPR-Cas9 has been used to disrupt the peptidylprolyl isomerase A gene, encoding cyclophilin A (CYPA), a protein that inhibits replication of cell culture-adapted HEV. In addition, hESC were modified to rescue expression of CYPA before terminal differentiation to HLCs and infection with HEV. It has been shown that the cells are permissive for infection by HEV and exhibited a replication-dependent type III interferon response. Thus, HLC provide a suitable tool to study HEV infection and replication *in vitro* and might offer new options for the development of antiviral drug candidates.

Recently, NTCP has been identified as a major receptor for HBV infections and blocking of the receptor–virus interaction might be a promising strategy for HBV infection treatment [198]. Further, binding of the

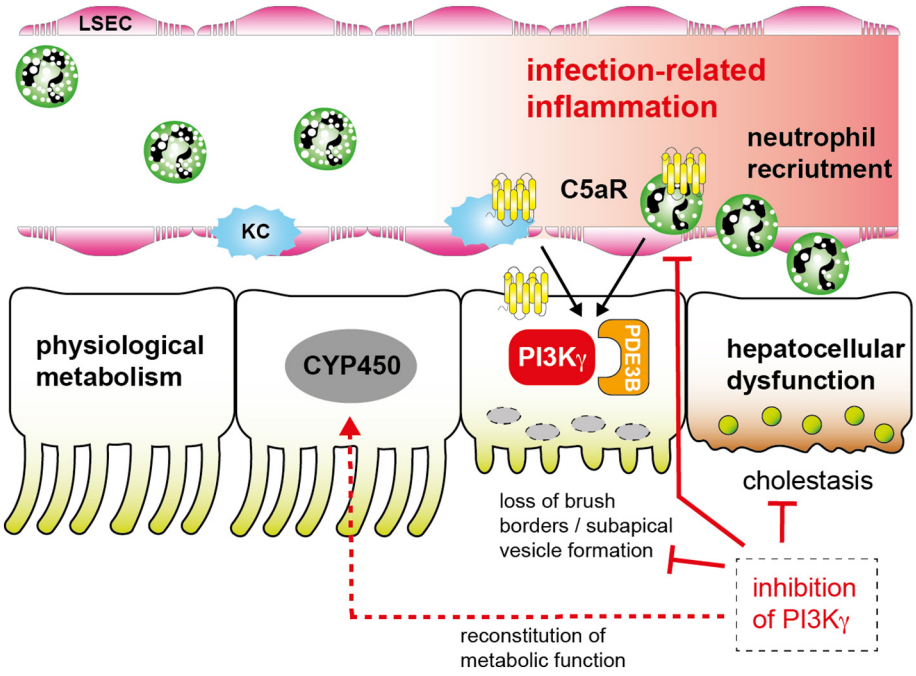

Fig. 5. Excretory dysfunction of the liver in systemic infection depends on PI3Kγ. The localized hepatic inflammatory milieu resulting from systemic infection/sepsis depends on paracrine and autocrine effects of hepatocytes and sinusoidal lining cells in response to PAMPs and circulating inflammatory mediators, where kinase-dependent and kinase-independent (through scaffolding of Phosphodiesterase 3B, PDE3B) activities of PI3Kγ play a significant role in mediating hepatocellular excretory failure. This phenotype is characterized by loss of brush borders (microvilli) that are reversibly incorporated into the hepatocyte to form subapical vesicles. Among the many important functions of PI3Kγ in the context of infection, recruitment of neutrophils is exquisitely dependent on this signaling pathway.

virus to highly sulphated HSPG appears to be required for infection [199]. It's worth noting, that already very low numbers of HBV particles (<10) are sufficient for effective infection of hepatocytes [200, 201]. Recent works indicates that scavenging of the virus by LSEC and transcytosis might play a role in helping the virus to cross the sinusoidal barrier. A mandatory initial step of LSEC by HBV might also explain the discrepancies between the high efficiency in liver targeting and the inefficient uptake of virus by cultured hepatocytes [199, 201, 202].

Hepatitis C virus (HCV) belongs to the most frequent diseases with up to 103 million people infected worldwide [203]. Chronic HCV infection causes jaundice-like symptoms and could result in the development of liver cirrhosis and hepatocellular carcinoma (HCC), which is one of the most prevalent primary malignant tumors that accounts for about 90% of all primary liver cancers. Despite the availability of effective new direct-acting antiviral drugs (DAA's), observed HCC recurrence rate still remains challenging [204]. Due to the lack of suitable models, our understanding of how hepatotropic viruses target the liver *in vivo* is less detailed. HCV attaches to HSPGs and subsequently interacts in a coordinated multi-step process to host cell receptors including low-density lipoprotein (LDL) receptor, scavenger receptor B1, CD81 and epidermal growth factor receptor (EGFR) on the hepatocyte surface, but also binds tight junction proteins claudin 1 and occludin before being endocytosed [205–210]. However, also for HCV it remains still unclear how the virus is specifically targeting the liver, as none of these receptors is expressed exclusively by hepatocytes. Within the host cell HCV envelope proteins E1 and E2 are known to form complexes with lipoproteins as part of the virus replication cycle. These "lipoviroparticles" are then subsequently released from infected cells as new HCV particles [211]. Viral infection of HCV was recently established in HLCs. Detection of HCV genome and HCV protease activity could confirmed an ongoing replication of the virus along with an innate immune response during infection that was not observed in carcinogenic cell lines [191]. Similarly, infection of HBV was supported by HLCs at late stage differentiation and could be maintained for several weeks with an innate immune response inhibitor [163]. Yoshida et al. recently showed that HLCs infected with adenovirus expressing SOX17, HEX and HNF4alpha are expressing all receptors required for virus entry and were permissive to pseudoparticles [212]. These findings were confirmed by two other studies in which HLCs supported the entire HCV viral replication cycle [191, 213].

4.2.2. Bacterial and fungal infection

Staphylococcus (*S.*) *aureus* bacteremia is one of the most common serious bacterial infections worldwide. In the United States of America, *S. aureus* was the most common bacterial isolate and accounts for 23% of all blood stream infections [214]. Most bacteria that reach the liver through the blood stream are recognized and efficiently cleared by tissue resident KC to prevent them from accessing hepatocytes under physiological conditions. However, at the liver sinusoid the pathogen causes formation of neutrophil extracellular traps (NET) that are associated with profound liver injury [215]. After crossing the sinusoidal barrier of the parenchymal compartment the pathogen is more difficult to eradicate and may even be facilitated in its persistence through the immune tolerogenic properties of the local microenvironment [195]. In an effort to prevent chronic inflammation caused by bacterial components of commensals from the gut, the liver favors a tolerogenic response towards incoming antigens [68, 69]. Consequently, KC respond to bacterial stimuli by secretion of anti-inflammatory cytokines [216], a phenomenon termed portal vein tolerance [217, 218]. Deregulation of an adapted immune response of the liver during systemic infection with *S. aureus* contributes to a rapid enrichment of bacteria within the liver. It has been recently shown, that a specific macrophage subset, liver capsular macrophages (LCM) replenished from blood monocytes, is an important regulator of granulocyte recruitment required for efficient clearance of pathogens during bacterial infections. Depletion of LCM in the liver decreased neutrophil recruitment and increased liver pathogen load [219]. However, due to the lack of suitable models a detailed analysis of the spatiotemporal regulation of cell recruitment in the course of infection and the factors involved in adherence and migration of circulating immune cells remain elusive. Although bacterial persistence within the liver is rather a rare event, liver infections are reported from mycobacteria and *Listeria* spp. that can establish granulomas [220, 221]. Mycobacteria could cause granuloma by infection of

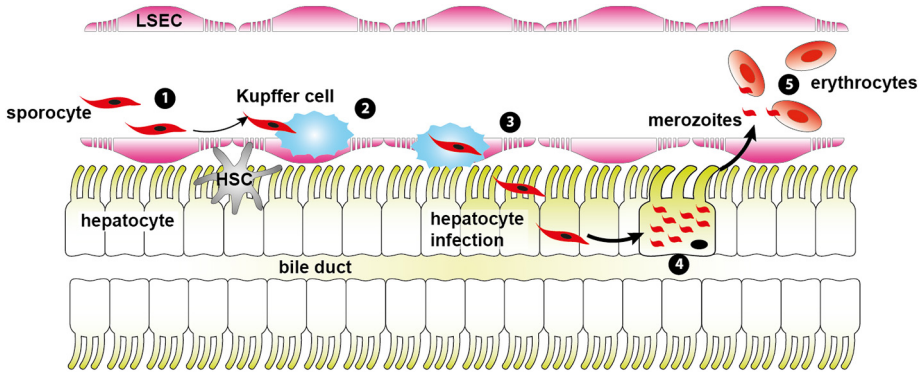

Fig. 6. Life cycle of Plamodium spp. infection in the liver. (1) A sporozoite enters the sinusoid, adheres to the sinusoidal endothelium and (2) glides to the next Kupffer cell. (3) It passes through the Kupffer cell and enters a hepatocyte. (4) After transmigrating through several hepatocytes, the sporozoite settles down in one final hepatocyte. The young liver stage parasite begins to grow, gradually displacing the organelles of the host hepatocyte. Infected hepatocytes eventually rupture and release merozoites into the sinusoid. (5) Subsequently merozoites can infect erythrocytes.

macrophages and secretion of bacterial proteins that induce the expression of matrix metalloproteinase 9 (MMP9) and subsequent tissue remodeling required for granuloma formation [222, 223]. It has been observed that granulomas could also provide a distinct anatomical compartment within the liver that supports the survival of bacteria. Granuloma can create a unique environment that is separated from the surrounding non-infected tissue and that acts as reservoir for dissemination virulent bacteria [220, 223]. Also *Francisella* (*F.*) *tularensis*, a gram-negative bacterium causing the zoonotic disease tularemia [224], has been shown to effect liver function upon infection. The bacteria primarily infects and persists within macrophages to evade primary immune detection [225] and several morphological alterations of the liver tissue have been observed in the progression of tularemia [226]. In a co-culture model comprising macrophages, hepatocytes and leukocytes we could recently show that *F. tularensis* replication in macrophages does not only prevent detection by immune cells but also facilitates subsequent infection of hepatocytes [227].

Fungal infections of the liver are most commonly caused by *Candida* spp. and often occur in immunocompromised patients. The main origin of *Candida* dissemination is thought to be colonization of the gastrointestinal tract. Invasive candidiasis, mainly caused by *Candida* (*C.*) *albicans*, is the sixth leading cause of nosocomial bloodstream infections in Europe [228] and associated with a mortality >40% despite the use of potent antifungal therapy [229]. In the course of infection, *C. albicans* is cleared from liver and spleen, whereas the fungal burden in the kidney increases. The ability to switch from yeast to hyphal growth forms (filamentation) is thought to be an essential aspect of *C. albicans* virulence as it renders fungal adhesion, invasion, escape from phagocytes, tissue damage and dissemination. However, the molecular mechanisms and enabling an adaption of fungi to the organ-specific environment are not fully understood. Liver MPS would offer a new approach to characterize virulence factors required for entry of the pathogen into the host cells and could provide new insight into the metabolic adaptions of the pathogens that enables its persistence within liver cells. Cellular targets could be identified and alterations of host cell signaling pathways investigated in more detail to allow the establishment of tailored cell targeting strategies, *i.e.* for cell type specific delivery of antibiotics to infected cells by encapsulation within functionalized nanocarriers [230–232].

4.2.3. Malaria

Plasmodium spp. infections have been intensively studied in the recent years. However, the invasion mechanisms malaria sporozoites use from the mosquito bite site to the final hepatocyte are not well understood. It is known, that parasites are delivered into the skin by a mosquito bite and avoid clearance by phagocytic cells through the rapid migration of sporozoites that enter lymphatics and blood vessels. The sporozoites than rapidly reach the liver and adhere to heparan sulphate proteoglycans (HSPGs) in the liver sinusoids. Tolerance induction to sporozoite antigens helps explain the poor, short-lasting protective immune response against liver stage malaria. Recent studies indicate that the sporozoites subsequently infiltrate phagocytic KC to overcome the sinusoidal barrier [233] and ultimately infect hepatocytes [234, 235]. Whereas it is known that KC passage occurs inside a vacuole [236] and sporozoite transmigration involves breaching of the hepatocyte membrane [237], entry into the final hepatocyte is a poorly documented step. Inside the final hepatocyte, the parasites develop into merozoites that are able to infect erythrocytes after rupture of the host cell [238] (Fig. 6).

Plasmodium infection was already modeled in HLC. In general, the cells were more susceptible to infection with increasing state of cell differentiation [194]. Late stage differentiated HLC were able to maintain sporozoite infection and expressed sufficient levels of cytochrom P450 enzymes to enable drug metabolism of *i.e.* primaquine, a drug frequently used to prevent persistence and replication of the parasite. The number of HLCs infected with *Plasmodium* varied between 10 and 60%. However, infection of erythrocytes from merozoites released from the HLCs cultures has not yet been demonstrated. Further, a stable long-term expression of host factors required for efficient infection and replication of the pathogens such as CD81, SRB1 (*Plasmodia* spp.) [194], or CLDN1 and NTCP (HCV, HBV infections) [163, 191] is still remains a challenge in hiPSC-derived liver tissues. Complex *in vitro* models, emulating all major cell types and tissues affected during journey of the parasite into the liver are required to gain a deeper insight into cell type and tissue specific pathways that are involved in the infection process. MPS would enable the investigation of the whole infection process by emulating the blood compartment, including immune cells and erythrocytes, in combination with hiPSC-derived liver tissues.

5. Conclusions and further perspectives

So far, hiPSC-derived cells of the liver have not been broadly employed for disease modeling in MPS. In fact, there is only one system we are aware of, a heart-liver-vascular platform (HeLiVa), operating with multiple co-cultures and perfusion [239]. In a matrix supported microchannel architecture vascularized by endothelial cells this system combines iPSC-derived cardiomyocytes with either PHH or iPSC-derived hepatocytes. Microtissue droplets were formed from HLCs co-cultured with fibroblasts and embedded in photopolymerized hydrogels assembled around an artificial vasculature. The application of multi-organ-on-chip platforms will certainly be the next important step in *in vitro* research on infectious diseases. Due to the increasing problem of multi-resistant bacteria able to cause infection to the liver [240] and emerging new pathogens causing metastatic infections to the liver such as *Streptococcus bovis* [241] and *Klebsiella pneumoniae* [242] due to systemic spread, more complex and reliable *in vitro* models emulating also systemic interactions of the liver with other organs are urgently needed. First multi organ-on-a-chip models were already described that

model the interaction of the liver with the lung and fat tissue to allow the investigation of drug biodistribution by microfluidic flow linkage [243]. The interaction between gut and liver was also mimicked by Esch et al. that constructed a liver-intestine biochip [244]. Bricks et al. also emulated the gut-liver axis by interconnecting cell-culture inserts in microfluidic biochips containing HepG2/C3A and Caco-2 cells, respectively [245]. In addition to the intestine, interaction of the liver with other organs such as kidney [246], neural cells [247] and skin has also been modeled [248].

Many molecular and cellular pathways involved in pathogen entry into the host cell of different organs, the mechanisms supporting pathogen persistence, and triggers required for the induction of post-infective organ regeneration still need to be uncovered. These questions could also be effectively addressed in complex *in vitro* models such as MPS to elucidate the underlying pathophysiology and to develop novel treatment options for critically ill patients. With the broader availability of more reliable *in vitro* systems we will also be able to further reduce the number of required animal experimentations in infection research. Several concerns were raised regarding the reliability of animal-tested products on humans which imposed ethical and economic pressures on pharmaceutical industries to amend their drug development methods [249]. MPS represent a powerful alternative to complement animal models as part of a tailored research strategies and could contribute to reduce animal numbers in biomedical research. Further, these systems can contribute to speed up the translation process in drug research from the nonclinical phase to clinical testing. MPS can even contribute to make translational research safer for the patient by using patient-specific stem cells and tissue derived therefrom to detect, *i.e.* idiosyncratic drug reactions in the context of a personalized medicine.

Acknowledgement

We acknowledge financial support from the Federal Ministry of Education and Research (grant no. 01EK1612B and 01EO1002), the German Research Foundation (grant no. MO 2968/1-1 to AM, and CRC PolyTarget (SFB 1278, Project C03 to MB)), the German Federal Institute for Risk Assessment (grant no. 1328-511 to AM), the German Research Platform for Zoonoses (grant no. 01KI1109 to AM), the Federal Construction Bank Thuringia (grant no. 2011 VF 0005 to AM) and the Center for Sepsis Control and Care, Jena (grant no. G4.3).

References

[1] F. Heymann, F. Tacke, Immunology in the liver—from homeostasis to disease, Nat. Rev. Gastroenterol. Hepatol. 13 (2016) 88–110.
[2] E. Maslak, A. Gregorius, S. Chlopicki, Liver sinusoidal endothelial cells (LSECs) function and NAFLD; NO-based therapy targeted to the liver, Pharmacol. Rep. 67 (2015) 689–694.
[3] J. Schlepper-Schafer, D. Hulsmann, A. Djovkar, H.E. Meyer, L. Herbertz, H. Kolb, V. Kolb-Bachofen, Endocytosis via galactose receptors in vivo. Ligand size directs uptake by hepatocytes and/or liver macrophages, Exp. Cell Res. 165 (1986) 494–506.
[4] J. Poisson, S. Lemoinne, C. Boulanger, F. Durand, R. Moreau, D. Valla, P.E. Rautou, Liver sinusoidal endothelial cells: physiology and role in liver diseases, J. Hepatol. 66 (2017) 212–227.
[5] B. Smedsrod, Clearance function of scavenger endothelial cells, Comp Hepatol 1 (3 Suppl) (2004) S22.
[6] P.A. McCourt, B. Hansen, D. Svistuonov, S. Johansson, P. Longati, K. Schledzewski, J. Kzhyshkowska, S. Goerdt, S. Johansson, B. Smedsrod, The liver sinusoidal endothelial cell hyaluronan receptor and its homolog, stabilin-1 - their roles (known and unknown) in endocytosis, Comp. Hepatol. 1 (3 Suppl) (2004) S24.
[7] P.A. Knolle, D. Wohlleber, Immunological functions of liver sinusoidal endothelial cells, Cell. Mol. Immunol. 13 (2016) 347–353.
[8] A. Schurich, J.P. Bottcher, S. Burgdorf, P. Penzler, S. Hegenbarth, M. Kern, A. Dolf, E. Endl, J. Schultze, E. Wiertz, D. Stabenow, C. Kurts, P. Knolle, Distinct kinetics and dynamics of cross-presentation in liver sinusoidal endothelial cells compared to dendritic cells, Hepatology 50 (2009) 909–919.
[9] A.M. Steffan, J.L. Gendrault, R.S. McCuskey, P.A. McCuskey, A. Kirn, Phagocytosis, an unrecognized property of murine endothelial liver cells, Hepatology 6 (1986) 830–836.
[10] L. Falasca, A. Bergamini, A. Serafino, C. Balabaud, L. Dini, Human Kupffer cell recognition and phagocytosis of apoptotic peripheral blood lymphocytes, Exp. Cell Res. 224 (1996) 152–162.
[11] L. Dini, P. Pagliara, E.C. Carla, Phagocytosis of apoptotic cells by liver: a morphological study, Microsc. Res. Tech. 57 (2002) 530–540.
[12] M.L. Balmer, E. Slack, A. de Gottardi, M.A. Lawson, S. Hapfelmeier, L. Miele, A. Grieco, H. Van Vlierberghe, R. Fahrner, N. Patuto, C. Bernsmeier, F. Ronchi, M. Wyss, D. Stroka, N. Dickgreber, M.H. Heim, K.D. McCoy, A.J. Macpherson, The liver may act as a firewall mediating mutualism between the host and its gut commensal microbiota, Sci. Transl. Med. 6 (2014) 237ra266.
[13] L. Bouwens, M. Baekeland, R. De Zanger, E. Wisse, Quantitation, tissue distribution and proliferation kinetics of Kupffer cells in normal rat liver, Hepatology 6 (1986) 718–722.
[14] M. Bauer, A.T. Press, M. Trauner, The liver in sepsis: patterns of response and injury, Curr. Opin. Crit. Care 19 (2013) 123–127.
[15] E. Seki, S. De Minicis, C.H. Osterreicher, J. Kluwe, Y. Osawa, D.A. Brenner, R.F. Schwabe, TLR4 enhances TGF-beta signaling and hepatic fibrosis, Nat. Med. 13 (2007) 1324–1332.
[16] T.R. Billiar, R.D. Curran, D.L. Williams, P.H. Kispert, Liver nonparenchymal cells are stimulated to provide interleukin 6 for induction of the hepatic acute-phase response in endotoxemia but not in remote localized inflammation, Arch. Surg. 127 (1992) 31–36 (discussion 36-37).
[17] J.V. Castell, M.J. Gomez-Lechon, M. David, T. Hirano, T. Kishimoto, P.C. Heinrich, Recombinant human interleukin-6 (IL-6/BSF-2/HSF) regulates the synthesis of acute phase proteins in human hepatocytes, FEBS Lett. 232 (1988) 347–350.
[18] M.I. Guillen, M.J. Gomez-Lechon, T. Nakamura, J.V. Castell, The hepatocyte growth factor regulates the synthesis of acute-phase proteins in human hepatocytes: divergent effect on interleukin-6-stimulated genes, Hepatology 23 (1996) 1345–1352.
[19] A.W. Thomson, P.A. Knolle, Antigen-presenting cell function in the tolerogenic liver environment, Nat. Rev. Immunol. 10 (2010) 753–766.
[20] B. Wang, M. Trippler, R. Pei, M. Lu, R. Broering, G. Gerken, J.F. Schlaak, Toll-like receptor activated human and murine hepatic stellate cells are potent regulators of hepatitis C virus replication, J. Hepatol. 51 (2009) 1037–1045.
[21] J. Wu, Z. Meng, M. Jiang, R. Pei, M. Trippler, R. Broering, A. Bucchi, J.P. Sowa, U. Dittmer, D. Yang, M. Roggendorf, G. Gerken, M. Lu, J.F. Schlaak, Hepatitis B virus suppresses toll-like receptor-mediated innate immune responses in murine parenchymal and nonparenchymal liver cells, Hepatology 49 (2009) 1132–1140.
[22] M. Kern, A. Popov, K. Scholz, B. Schumak, D. Djandji, A. Limmer, D. Eggle, T. Sacher, R. Zawatzky, R. Holtappels, M.J. Reddehase, G. Hartmann, S. Debey-Pascher, L. Diehl, U. Kalinke, U. Koszinowski, J. Schultze, P.A. Knolle, Virally infected mouse liver endothelial cells trigger CD8+ T-cell immunity, Gastroenterology 138 (2010) 336–346.
[23] I.N. Crispe, Hepatocytes as immunological agents, J. Immunol. 196 (2016) 17–21.
[24] I.G. Rodrigue-Gervais, K. Labbe, M. Dagenais, J. Dupaul-Chicoine, C. Champagne, A. Morizot, A. Skeldon, E.L. Brincks, S.M. Vidal, T.S. Griffith, M. Saleh, Cellular inhibitor of apoptosis protein cIAP2 protects against pulmonary tissue necrosis during influenza virus infection to promote host survival, Cell Host Microbe 15 (2014) 23–35.
[25] L. Raberg, D. Sim, A.F. Read, Disentangling genetic variation for resistance and tolerance to infectious diseases in animals, Science 318 (2007) 812–814.
[26] R. Gozzelino, B.B. Andrade, R. Larsen, N.F. Luz, L. Vanoaica, E. Seixas, A. Coutinho, S. Cardoso, S. Rebelo, M. Poli, M. Barral-Netto, D. Darshan, L.C. Kuhn, M.P. Soares, Metabolic adaptation to tissue iron overload confers tolerance to malaria, Cell Host Microbe 12 (2012) 693–704.
[27] S. Weis, A.R. Carlos, M.R. Moita, S. Singh, B. Blankenhaus, S. Cardoso, R. Larsen, S. Rebelo, S. Schauble, L. Del Barrio, G. Mithieux, F. Rajas, S. Lindig, M. Bauer, M.P. Soares, Metabolic adaptation establishes disease tolerance to sepsis, Cell 169 (2017) 1263–1275 (e1214).
[28] S. Weis, I. Rubio, K. Ludwig, C. Weigel, E. Jentho, Hormesis and defense of infectious disease, Int. J. Mol. Sci. 18 (2017).
[29] I.N. Shalova, J.Y. Lim, M. Chittezhath, A.S. Zinkernagel, F. Beasley, E. Hernandez-Jimenez, V. Toledano, C. Cubillos-Zapata, A. Rapisarda, J. Chen, K. Duan, H. Yang, M. Poidinger, G. Melillo, V. Nizet, F. Arnalich, E. Lopez-Collazo, S.K. Biswas, Human monocytes undergo functional re-programming during sepsis mediated by hypoxia-inducible factor-1alpha, Immunity 42 (2015) 484–498.
[30] J. Claria, R.E. Stauber, M.J. Coenraad, R. Moreau, R. Jalan, M. Pavesi, A. Amoros, E. Titos, J. Alcaraz-Quiles, K. Oettl, M. Morales-Ruiz, P. Angeli, M. Domenicali, C. Alessandria, A. Gerbes, J. Wendon, F. Nevens, J. Trebicka, W. Laleman, F. Saliba, T. M. Welzel, A. Albillos, T. Gustot, D. Benten, F. Durand, P. Gines, M. Bernardi, V. Arroyo, C.S.I.o.t.E.-C. Consortium, F, The European foundation for the study of chronic liver, systemic inflammation in decompensated cirrhosis: characterization and role in acute-on-chronic liver failure, Hepatology 64 (2016) 1249–1264.
[31] D. Binda, D. Lasserre-Bigot, A. Bonet, M. Thomassin, M.P. Come, C. Guinchard, R. Bars, A. Jacqueson, L. Richert, Time course of cytochromes P450 decline during rat hepatocyte isolation and culture: effect of L-NAME, Toxicol. in Vitro 17 (2003) 59–67.
[32] J. Beigel, K. Fella, P.J. Kramer, M. Kroeger, P. Hewitt, Genomics and proteomics analysis of cultured primary rat hepatocytes, Toxicol. in Vitro 22 (2008) 171–181.
[33] A.M. Tormos, R. Talens-Visconti, A. Bonora-Centelles, S. Perez, J. Sastre, Oxidative stress triggers cytokinesis failure in hepatocytes upon isolation, Free Radic. Res. 49 (2015) 927–934.
[34] M. Vinken, P. Papeleu, S. Snykers, E. De Rop, T. Henkens, J.K. Chipman, V. Rogiers, T. Vanhaecke, Involvement of cell junctions in hepatocyte culture functionality, Crit. Rev. Toxicol. 36 (2006) 299–318.
[35] N.J. Hewitt, P. Hewitt, I. Phase, II enzyme characterization of two sources of HepG2 cell lines, Xenobiotica 34 (2004) 243–256.
[36] M.J. Evans, M.H. Kaufman, Establishment in culture of pluripotential cells from mouse embryos, Nature 292 (1981) 154–156.
[37] A. Raya, I. Rodriguez-Piza, B. Aran, A. Consiglio, P.N. Barri, A. Veiga, J.C. Izpisua Belmonte, Generation of cardiomyocytes from new human embryonic stem cell

lines derived from poor-quality blastocysts, Cold Spring Harb. Symp. Quant. Biol. 73 (2008) 127–135.
[38] Y. Chung, I. Klimanskaya, S. Becker, T. Li, M. Maserati, J. Lu, T. Zdravkovic, D. Ilic, O. Genbacev, S. Fisher, A. Krtolica, R. Lanza, Human embryonic stem cell lines generated without embryo destruction, Cell Stem Cell 2 (2008) 113–117.
[39] I. Klimanskaya, Embryonic stem cells from blastomeres maintaining embryo viability, Semin. Reprod. Med. 31 (2013) 49–55.
[40] K. Takahashi, K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda, S. Yamanaka, Induction of pluripotent stem cells from adult human fibroblasts by defined factors, Cell 131 (2007) 861–872.
[41] J.M. Wells, D.A. Melton, Vertebrate endoderm development, Annu. Rev. Cell Dev. Biol. 15 (1999) 393–410.
[42] D.C. Hay, D. Zhao, J. Fletcher, Z.A. Hewitt, D. McLean, A. Urruticoechea-Uriguen, J.R. Black, C. Elcombe, J.A. Ross, R. Wolf, W. Cui, Efficient differentiation of hepatocytes from human embryonic stem cells exhibiting markers recapitulating liver development in vivo, Stem Cells 26 (2008) 894–902.
[43] Y. Yu, H. Liu, Y. Ikeda, B.P. Amiot, P. Rinaldo, S.A. Duncan, S.L. Nyberg, Hepatocyte-like cells differentiated from human induced pluripotent stem cells: relevance to cellular therapies, Stem Cell Res. 9 (2012) 196–207.
[44] C. Payne, J. King, D. Hay, The role of activin/nodal and Wnt signaling in endoderm formation, Vitam. Horm. 85 (2011) 207–216.
[45] K.A. D'Amour, A.D. Agulnick, S. Eliazer, O.G. Kelly, E. Kroon, E.E. Baetge, Efficient differentiation of human embryonic stem cells to definitive endoderm, Nat. Biotechnol. 23 (2005) 1534–1541.
[46] A. Kubo, K. Shinozaki, J.M. Shannon, V. Kouskoff, M. Kennedy, S. Woo, H.J. Fehling, G. Keller, Development of definitive endoderm from embryonic stem cells in culture, Development 131 (2004) 1651–1662.
[47] A.T. Moerkamp, A. Paca, M.J. Goumans, T. Kunath, B.P.T. Kruithof, M. Kruithof-de Julio, Extraembryonic endoderm cells as a model of endoderm development, Develop. Growth Differ. 55 (2013) 301–308.
[48] M. Familari, Characteristics of the endoderm: embryonic and extraembryonic in mouse, ScientificWorldJournal 6 (2006) 1815–1827.
[49] A.B. McLean, K.A. D'Amour, K.L. Jones, M. Krishnamoorthy, M.J. Kulik, D.M. Reynolds, A.M. Sheppard, H. Liu, Y. Xu, E.E. Baetge, S. Dalton, Activin a efficiently specifies definitive endoderm from human embryonic stem cells only when phosphatidylinositol 3-kinase signaling is suppressed, Stem Cells 25 (2007) 29–38.
[50] L. Sui, L. Bouwens, J.K. Mfopou, Signaling pathways during maintenance and definitive endoderm differentiation of embryonic stem cells, Int. J. Dev. Biol. 57 (2013) 1–12.
[51] H. Ninomiya, K. Mizuno, R. Terada, T. Miura, K. Ohnuma, S. Takahashi, M. Asashima, T. Michiue, Improved efficiency of definitive endoderm induction from human induced pluripotent stem cells in feeder and serum-free culture system, In Vitro Cell. Dev. Biol. Anim. 51 (2015) 1–8.
[52] J.V. Schiesser, S.J. Micallef, S. Hawes, A.G. Elefanty, E.G. Stanley, Derivation of insulin-producing beta-cells from human pluripotent stem cells, Rev. Diabet. Stud. 11 (2014) 6–18.
[53] A.K. Teo, Y. Ali, K.Y. Wong, H. Chipperfield, A. Sadasivam, Y. Poobalan, E.K. Tan, S.T. Wang, S. Abraham, N. Tsuneyoshi, L.W. Stanton, N.R. Dunn, Activin and BMP4 synergistically promote formation of definitive endoderm in human embryonic stem cells, Stem Cells 30 (2012) 631–642.
[54] E. Hoveizi, M. Nabiuni, K. Parivar, J. Ai, M. Massumi, Definitive endoderm differentiation of human-induced pluripotent stem cells using signaling molecules and IDE1 in three-dimensional polymer scaffold, J. Biomed. Mater. Res. A 102 (2014) 4027–4036.
[55] O. Naujok, U. Diekmann, S. Lenzen, The generation of definitive endoderm from human embryonic stem cells is initially independent from activin A but requires canonical Wnt-signaling, Stem Cell Rev. 10 (2014) 480–493.
[56] L. Romorini, X. Garate, G. Neiman, C. Luzzani, V.A. Furmento, A.S. Guberman, G.E. Sevlever, M.E. Scassa, S.G. Miriuka, AKT/GSK3beta signaling pathway is critically involved in human pluripotent stem cell survival, Sci. Rep. 6 (2016) 35660.
[57] L. Vallier, M. Alexander, R.A. Pedersen, Activin/nodal and FGF pathways cooperate to maintain pluripotency of human embryonic stem cells, J. Cell Sci. 118 (2005) 4495–4509.
[58] T. Sumi, N. Tsuneyoshi, N. Nakatsuji, H. Suemori, Defining early lineage specification of human embryonic stem cells by the orchestrated balance of canonical Wnt/beta-catenin, activin/nodal and BMP signaling, Development 135 (2008) 2969–2979.
[59] A.M. Hossini, A.S. Quast, M. Plotz, K. Grauel, T. Exner, J. Kuchler, H. Stachelscheid, J. Eberle, A. Rabien, E. Makrantonaki, C.C. Zouboulis, PI3K/AKT signaling pathway is essential for survival of induced pluripotent stem cells, PLoS One 11 (2016), e0154770.
[60] J.C. Smith, B.M. Price, K. Van Nimmen, D. Huylebroeck, Identification of a potent *Xenopus* mesoderm-inducing factor as a homologue of activin A, Nature 345 (1990) 729–731.
[61] L.W. Gamer, C.V. Wright, Autonomous endodermal determination in *Xenopus*: regulation of expression of the pancreatic gene XlHbox 8, Dev. Biol. 171 (1995) 240–251.
[62] K.A. D'Amour, A.G. Bang, S. Eliazer, O.G. Kelly, A.D. Agulnick, N.G. Smart, M.A. Moorman, E. Kroon, M.K. Carpenter, E.E. Baetge, Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells, Nat. Biotechnol. 24 (2006) 1392–1401.
[63] J.K. Mfopou, M. Geeraerts, R. Dejene, S. Van Langenhoven, A. Aberkane, L.A. Van Grunsven, L. Bouwens, Efficient definitive endoderm induction from mouse embryonic stem cell adherent cultures: a rapid screening model for differentiation studies, Stem Cell Res. 12 (2014) 166–177.
[64] K.G. Chen, B.S. Mallon, R.D. McKay, P.G. Robey, Human pluripotent stem cell culture: considerations for maintenance, expansion, and therapeutics, Cell Stem Cell 14 (2014) 13–26.
[65] M. Ohgushi, Y. Sasai, Lonely death dance of human pluripotent stem cells: ROCKing between metastable cell states, Trends Cell Biol. 21 (2011) 274–282.
[66] Y. Komiya, R. Habas, Wnt signal transduction pathways, Organ 4 (2008) 68–75.
[67] T. Ishizaki, M. Uehata, I. Tamechika, J. Keel, K. Nonomura, M. Maekawa, S. Narumiya, Pharmacological properties of Y-27632, a specific inhibitor of rho-associated kinases, Mol. Pharmacol. 57 (2000) 976–983.
[68] K. Watanabe, M. Ueno, D. Kamiya, A. Nishiyama, M. Matsumura, T. Wataya, J.B. Takahashi, S. Nishikawa, S. Nishikawa, K. Muguruma, Y. Sasai, A ROCK inhibitor permits survival of dissociated human embryonic stem cells, Nat. Biotechnol. 25 (2007) 681–686.
[69] M. Maldonado, R.J. Luu, M.E. Ramos, J. Nam, ROCK inhibitor primes human induced pluripotent stem cells to selectively differentiate towards mesendodermal lineage via epithelial-mesenchymal transition-like modulation, Stem Cell Res. 17 (2016) 222–227.
[70] H. Lee, C. Haller, C. Manneville, T. Doll, I. Fruh, C.G. Keller, S.M. Richards, Y. Ibig-Rehm, M. Patoor, M. Goette, L.C. Bouchez, M. Mueller, Identification of small molecules which induce skeletal muscle differentiation in embryonic stem cells via activation of the Wnt and inhibition of Smad2/3 and sonic hedgehog pathways, Stem Cells 34 (2016) 299–310.
[71] G.L. Engelmann, J.L. Staecker, A.G. Richardson, Effect of sodium butyrate on primary cultures of adult rat hepatocytes, In Vitro Cell. Dev. Biol. 23 (1987) 86–92.
[72] N.S. Sharma, R. Shikhanovich, R. Schloss, M.L. Yarmush, Sodium butyrate-treated embryonic stem cells yield hepatocyte-like cells expressing a glycolytic phenotype, Biotechnol. Bioeng. 94 (2006) 1053–1063.
[73] Q.J. Zhou, L.X. Xiang, J.Z. Shao, R.Z. Hu, Y.L. Lu, H. Yao, L.C. Dai, In vitro differentiation of hepatic progenitor cells from mouse embryonic stem cells induced by sodium butyrate, J. Cell. Biochem. 100 (2007) 29–42.
[74] S. Snykers, T. Henkens, E. De Rop, M. Vinken, J. Fraczek, J. De Kock, E. De Prins, A. Geerts, V. Rogiers, T. Vanhaecke, Role of epigenetics in liver-specific gene transcription, hepatocyte differentiation and stem cell reprogrammation, J. Hepatol. 51 (2009) 187–211.
[75] A.J. Firestone, J.K. Chen, Controlling destiny through chemistry: small-molecule regulators of cell fate, ACS Chem. Biol. 5 (2010) 15–34.
[76] M. Kajiwara, T. Aoi, K. Okita, R. Takahashi, H. Inoue, N. Takayama, H. Endo, K. Eto, J. Toguchida, S. Uemoto, S. Yamanaka, Donor-dependent variations in hepatic differentiation from human-induced pluripotent stem cells, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 12538–12543.
[77] D.T. Peters, C.A. Henderson, C.R. Warren, M. Friesen, F. Xia, C.E. Becker, K. Musunuru, C.A. Cowan, Asialoglycoprotein receptor 1 is a specific cell-surface marker for isolating hepatocytes derived from human pluripotent stem cells, Development 143 (2016) 1475–1481.
[78] K. Si-Tayeb, F.K. Noto, M. Nagaoka, J. Li, M.A. Battle, C. Duris, P.E. North, S. Dalton, S. A. Duncan, Highly efficient generation of human hepatocyte-like cells from induced pluripotent stem cells, Hepatology 51 (2010) 297–305.
[79] H. Basma, A. Soto-Gutierrez, G.R. Yannam, L. Liu, R. Ito, T. Yamamoto, E. Ellis, S.D. Carson, S. Sato, Y. Chen, D. Muirhead, N. Navarro-Alvarez, R.J. Wong, J. Roy-Chowdhury, J.L. Platt, D.F. Mercer, J.D. Miller, S.C. Strom, N. Kobayashi, I.J. Fox, Differentiation and transplantation of human embryonic stem cell-derived hepatocytes, Gastroenterology 136 (2009) 990–999.
[80] N.R. Hannan, C.P. Segeritz, T. Touboul, L. Vallier, Production of hepatocyte-like cells from human pluripotent stem cells, Nat. Protoc. 8 (2013) 430–437.
[81] M. Ogawa, S. Ogawa, C.E. Bear, S. Ahmadi, S. Chin, B. Li, M. Grompe, G. Keller, B.M. Kamath, A. Ghanekar, Directed differentiation of cholangiocytes from human pluripotent stem cells, Nat. Biotechnol. 33 (2015) 853–861.
[82] J. Jung, M. Zheng, M. Goldfarb, K.S. Zaret, Initiation of mammalian liver development from endoderm by fibroblast growth factors, Science 284 (1999) 1998–2003.
[83] V. Gouon-Evans, L. Boussemart, P. Gadue, D. Nierhoff, C.I. Koehler, A. Kubo, D.A. Shafritz, G. Keller, BMP-4 is required for hepatic specification of mouse embryonic stem cell-derived definitive endoderm, Nat. Biotechnol. 24 (2006) 1402–1411.
[84] J.M. Rossi, N.R. Dunn, B.L. Hogan, K.S. Zaret, Distinct mesodermal signals, including BMPs from the septum transversum mesenchyme, are required in combination for hepatogenesis from the endoderm, Genes Dev. 15 (2001) 1998–2009.
[85] Y. Duan, A. Catana, Y. Meng, N. Yamamoto, S. He, S. Gupta, S.S. Gambhir, M.A. Zern, Differentiation and enrichment of hepatocyte-like cells from human embryonic stem cells in vitro and in vivo, Stem Cells 25 (2007) 3058–3068.
[86] A. Kamiya, T. Kinoshita, A. Miyajima, Oncostatin M and hepatocyte growth factor induce hepatic maturation via distinct signaling pathways, FEBS Lett. 492 (2001) 90–94.
[87] M. Yasunaga, S. Tada, S. Torikai-Nishikawa, Y. Nakano, M. Okada, L.M. Jakt, S. Nishikawa, T. Chiba, T. Era, S. Nishikawa, Induction and monitoring of definitive and visceral endoderm differentiation of mouse ES cells, Nat. Biotechnol. 23 (2005) 1542–1550.
[88] R. Siller, S. Greenhough, E. Naumovska, G.J. Sullivan, Small-molecule-driven hepatocyte differentiation of human pluripotent stem cells, Stem Cell Rep. 4 (2015) 939–952.
[89] P.H. Weigel, J.A. Oka, The surface content of asialoglycoprotein receptors on isolated hepatocytes is reversibly modulated by changes in temperature, J. Biol. Chem. 258 (1983) 5089–5094.
[90] X. Ma, Y. Duan, B. Tschudy-Seney, G. Roll, I.S. Behbahan, T.P. Ahuja, V. Tolstikov, C. Wang, J. McGee, S. Khoobyari, J.A. Nolta, H. Willenbring, M.A. Zern, Highly efficient differentiation of functional hepatocytes from human induced pluripotent stem cells, Stem Cells Transl. Med. 2 (2013) 409–419.
[91] K.E. McGrath, A.D. Koniski, K.M. Maltby, J.K. McGann, J. Palis, Embryonic expression and function of the chemokine SDF-1 and its receptor, CXCR4, Dev. Biol. 213 (1999) 442–456.
[92] R.G. Ireland, C.A. Simmons, Human pluripotent stem cell mechanobiology: manipulating the biophysical microenvironment for regenerative medicine and tissue engineering applications, Stem Cells 33 (2015) 3187–3196.
[93] Y. Sun, C.S. Chen, J. Fu, Forcing stem cells to behave: a biophysical perspective of the cellular microenvironment, Annu. Rev. Biophys. 41 (2012) 519–542.

[94] L.B. Hazeltine, J.A. Selekman, S.P. Palecek, Engineering the human pluripotent stem cell microenvironment to direct cell fate, Biotechnol. Adv. 31 (2013) 1002–1019.
[95] R. Kojima, K. Yoshimoto, E. Takahashi, M. Ichino, H. Miyoshi, Y. Nagasaki, Spheroid array of fetal mouse liver cells constructed on a PEG-gel micropatterned surface: upregulation of hepatic functions by co-culture with nonparenchymal liver cells, Lab Chip 9 (2009) 1991–1993.
[96] C. Duret, S. Gerbal-Chaloin, J. Ramos, J.M. Fabre, E. Jacquet, F. Navarro, P. Blanc, A. Sa-Cunha, P. Maurel, M. Daujat-Chavanieu, Isolation, characterization, and differentiation to hepatocyte-like cells of nonparenchymal epithelial cells from adult human liver, Stem Cells 25 (2007) 1779–1790.
[97] A. Soto-Gutierrez, N. Navarro-Alvarez, D. Zhao, J.D. Rivas-Carrillo, J. Lebkowski, N. Tanaka, I.J. Fox, N. Kobayashi, Differentiation of mouse embryonic stem cells to hepatocyte-like cells by co-culture with human liver nonparenchymal cell lines, Nat. Protoc. 2 (2007) 347–356.
[98] C. Kordes, I. Sawitza, S. Gotze, D. Herebian, D. Haussinger, Hepatic stellate cells contribute to progenitor cells and liver regeneration, J. Clin. Invest. 124 (2014) 5503–5515.
[99] M.B. Esch, J.M. Prot, Y.I. Wang, P. Miller, J.R. Llamas-Vidales, B.A. Naughton, D.R. Applegate, M.L. Shuler, Multi-cellular 3D human primary liver cell culture elevates metabolic activity under fluidic flow, Lab Chip 15 (2015) 2269–2277.
[100] N. Tuleuova, J.Y. Lee, J. Lee, E. Ramanculov, M.A. Zern, A. Revzin, Using growth factor arrays and micropatterned co-cultures to induce hepatic differentiation of embryonic stem cells, Biomaterials 31 (2010) 9221–9231.
[101] Y. Koui, T. Kido, T. Ito, H. Oyama, S.W. Chen, Y. Katou, K. Shirahige, A. Miyajima, An in vitro human liver model by iPSC-derived parenchymal and non-parenchymal cells, Stem Cell Rep. 9 (2017) 490–498.
[102] C. Takagi, H. Yagi, M. Hieda, K. Tajima, T. Hibi, Y. Abe, M. Kitago, M. Shinoda, O. Itano, Y. Kitagawa, Mesenchymal stem cells contribute to hepatic maturation of human induced pluripotent stem cells, Eur. Surg. Res. 58 (2017) 27–39.
[103] Y. Kadota, H. Yagi, K. Inomata, K. Matsubara, T. Hibi, Y. Abe, M. Kitago, M. Shinoda, H. Obara, O. Itano, Y. Kitagawa, Mesenchymal stem cells support hepatocyte function in engineered liver grafts, Organ 10 (2014) 268–277.
[104] Y. Nagamoto, K. Tashiro, K. Takayama, K. Ohashi, K. Kawabata, F. Sakurai, M. Tachibana, T. Hayakawa, M.K. Furue, H. Mizuguchi, The promotion of hepatic maturation of human pluripotent stem cells in 3D co-culture using type I collagen and Swiss 3T3 cell sheets, Biomaterials 33 (2012) 4526–4534.
[105] B. Wang, A.E. Jakus, P.M. Baptista, S. Soker, A. Soto-Gutierrez, M.M. Abecassis, R.N. Shah, J.A. Wertheim, Functional maturation of induced pluripotent stem cell hepatocytes in extracellular matrix-a comparative analysis of bioartificial liver microenvironments, Stem Cells Transl. Med. 5 (2016) 1257–1267.
[106] F. Meier, N. Freyer, J. Brzeszczynska, F. Knospel, L. Armstrong, M. Lako, S. Greuel, G. Damm, E. Ludwig-Schwellinger, U. Deschl, J.A. Ross, M. Beilmann, K. Zeilinger, Hepatic differentiation of human iPSCs in different 3D models: a comparative study, Int. J. Mol. Med. 40 (2017) 1759–1771.
[107] H. Baharvand, S.M. Hashemi, S. Kazemi Ashtiani, A. Farrokhi, Differentiation of human embryonic stem cells into hepatocytes in 2D and 3D culture systems in vitro, Int. J. Dev. Biol. 50 (2006) 645–652.
[108] D.R. Berger, B.R. Ware, M.D. Davidson, S.R. Allsup, S.R. Khetani, Enhancing the functional maturity of induced pluripotent stem cell-derived human hepatocytes by controlled presentation of cell-cell interactions in vitro, Hepatology 61 (2015) 1370–1381.
[109] R.L. Gieseck 3rd, N.R. Hannan, R. Bort, N.A. Hanley, R.A. Drake, G.W. Cameron, T.A. Wynn, L. Vallier, Maturation of induced pluripotent stem cell derived hepatocytes by 3D-culture, PLoS One 9 (2014), e86372.
[110] X. Ma, X. Qu, W. Zhu, Y.S. Li, S. Yuan, H. Zhang, J. Liu, P. Wang, C.S. Lai, F. Zanella, G.S. Feng, F. Sheikh, S. Chien, S. Chen, Deterministically patterned biomimetic human iPSC-derived hepatic model via rapid 3D bioprinting, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) 2206–2211.
[111] P. Godoy, N.J. Hewitt, U. Albrecht, M.E. Andersen, N. Ansari, S. Bhattacharya, J.G. Bode, J. Bolleyn, C. Borner, J. Bottger, A. Braeuning, R.A. Budinsky, B. Burkhardt, N.R. Cameron, G. Camussi, C.S. Cho, Y.J. Choi, J. Craig Rowlands, U. Dahmen, G. Damm, O. Dirsch, M.T. Donato, J. Dong, S. Dooley, D. Drasdo, R. Eakins, K.S. Ferreira, V. Fonsato, J. Fraczek, R. Gebhardt, A. Gibson, M. Glanemann, C.E. Goldring, M.J. Gomez-Lechon, G.M. Groothuis, L. Gustavsson, C. Guyot, D. Hallifax, S. Hammad, A. Hayward, D. Haussinger, C. Hellerbrand, P. Hewitt, S. Hoehme, H.G. Holzhutter, J.B. Houston, J. Hrach, K. Ito, H. Jaeschke, V. Keitel, J.M. Kelm, B. Kevin Park, C. Kordes, G.A. Kullak-Ublick, E.L. LeCluyse, P. Lu, J. Luebke-Wheeler, A. Lutz, D.J. Maltman, M. Matz-Soja, P. McMullen, I. Merfort, S. Messner, C. Meyer, J. Mwinyi, D.J. Naisbitt, A.K. Nussler, P. Olinga, F. Pampaloni, J. Pi, L. Pluta, S.A. Przyborski, A. Ramachandran, V. Rogiers, C. Rowe, C. Schelcher, K. Schmich, M. Schwarz, B. Singh, E.H. Stelzer, B. Stieger, R. Stober, Y. Sugiyama, C. Tetta, W.E. Thasler, T. Vanhaecke, M. Vinken, T.S. Weiss, A. Widera, C.G. Woods, J.J. Xu, K.M. Yarborough, J.G. Hengstler, Recent advances in 2D and 3D in vitro systems using primary hepatocytes, alternative hepatocyte sources and non-parenchymal liver cells and their use in investigating mechanisms of hepatotoxicity, cell signaling and ADME, Arch. Toxicol. 87 (2013) 1315–1530.
[112] C. Du, K. Narayanan, M.F. Leong, A.C. Wan, Induced pluripotent stem cell-derived hepatocytes and endothelial cells in multi-component hydrogel fibers for liver tissue engineering, Biomaterials 35 (2014) 6006–6014.
[113] X. Zhan, G. Dravid, Z. Ye, H. Hammond, M. Shamblott, J. Gearhart, L. Cheng, Functional antigen-presenting leucocytes derived from human embryonic stem cells in vitro, Lancet 364 (2004) 163–171.
[114] A. Chhabra, I.P. Chen, D. Batra, Human dendritic cell-derived induced pluripotent stem cell lines are not immunogenic, J. Immunol. 198 (2017) 1875–1886.
[115] M. Themeli, C.C. Kloss, G. Ciriello, V.D. Fedorov, F. Perna, M. Gonen, M. Sadelain, Generation of tumor-targeted human T lymphocytes from induced pluripotent stem cells for cancer therapy, Nat. Biotechnol. 31 (2013) 928–933.
[116] T. Nishimura, S. Kaneko, A. Kawana-Tachikawa, Y. Tajima, H. Goto, D. Zhu, K. Nakayama-Hosoya, S. Iriguchi, Y. Uemura, T. Shimizu, N. Takayama, D. Yamada, K. Nishimura, M. Ohtaka, N. Watanabe, S. Takahashi, A. Iwamoto, H. Koseki, M. Nakanishi, K. Eto, H. Nakauchi, Generation of rejuvenated antigen-specific T cells by reprogramming to pluripotency and redifferentiation, Cell Stem Cell 12 (2013) 114–126.
[117] R. Vizcardo, K. Masuda, D. Yamada, T. Ikawa, K. Shimizu, S. Fujii, H. Koseki, H. Kawamoto, Regeneration of human tumor antigen-specific T cells from iPSCs derived from mature CD8(+) T cells, Cell Stem Cell 12 (2013) 31–36.
[118] D.A. Knorr, D.S. Kaufman, Pluripotent stem cell-derived natural killer cells for cancer therapy, Transl. Res. 156 (2010) 147–154.
[119] C. Eguizabal, O. Zenarruzabeitia, J. Monge, S. Santos, M.A. Vesga, N. Maruri, A. Arrieta, M. Rinon, E. Tamayo-Orbegozo, L. Amo, S. Larrucea, F. Borrego, Natural killer cells for cancer immunotherapy: pluripotent stem cells-derived NK cells as an immunotherapeutic perspective, Front. Immunol. 5 (2014) 439.
[120] P.S. Becker, G. Suck, P. Nowakowska, E. Ullrich, E. Seifried, P. Bader, T. Tonn, C. Seidl, Selection and expansion of natural killer cells for NK cell-based immunotherapy, Cancer Immunol. Immunother. 65 (2016) 477–484.
[121] P.S. Woll, C.H. Martin, J.S. Miller, D.S. Kaufman, Human embryonic stem cell-derived NK cells acquire functional receptors and cytolytic activity, J. Immunol. 175 (2005) 5095–5103.
[122] P.S. Woll, B. Grzywacz, X. Tian, R.K. Marcus, D.A. Knorr, M.R. Verneris, D.S. Kaufman, Human embryonic stem cells differentiate into a homogeneous population of natural killer cells with potent in vivo antitumor activity, Blood 113 (2009) 6094–6101.
[123] S. Senju, M. Haruta, K. Matsumura, Y. Matsunaga, S. Fukushima, T. Ikeda, K. Takamatsu, A. Irie, Y. Nishimura, Generation of dendritic cells and macrophages from human induced pluripotent stem cells aiming at cell therapy, Gene Ther. 18 (2011) 874–883.
[124] S. Senju, M. Haruta, Y. Matsunaga, S. Fukushima, T. Ikeda, K. Takahashi, K. Okita, S. Yamanaka, Y. Nishimura, Characterization of dendritic cells and macrophages generated by directed differentiation from mouse induced pluripotent stem cells, Stem Cells 27 (2009) 1021–1031.
[125] M. Haruta, Y. Tomita, A. Yuno, K. Matsumura, T. Ikeda, K. Takamatsu, E. Haga, C. Koba, Y. Nishimura, S. Senju, TAP-deficient human iPS cell-derived myeloid cell lines as unlimited cell source for dendritic cell-like antigen-presenting cells, Gene Ther. 20 (2013) 504–513.
[126] A.T.Y. Yeung, C. Hale, A.H. Lee, E.E. Gill, W. Bushell, D. Parry-Smith, D. Goulding, D. Pickard, T. Roumeliotis, J. Choudhary, N. Thomson, W.C. Skarnes, G. Dougan, R.E.W. Hancock, Exploiting induced pluripotent stem cell-derived macrophages to unravel host factors influencing *Chlamydia trachomatis* pathogenesis, Nat. Commun. 8 (2017) 15013.
[127] N. Lachmann, M. Ackermann, E. Frenzel, S. Liebhaber, S. Brennig, C. Happle, D. Hoffmann, O. Klimenkova, D. Luttge, T. Buchegger, M.P. Kuhnel, A. Schambach, S. Janciauskiene, C. Figueiredo, G. Hansen, J. Skokowa, T. Moritz, Large-scale hematopoietic differentiation of human induced pluripotent stem cells provides granulocytes or macrophages for cell replacement therapies, Stem Cell Rep. 4 (2015) 282–296.
[128] K. Ren, J. Zhou, H. Wu, Materials for microfluidic chip fabrication, Acc. Chem. Res. 46 (2013) 2396–2406.
[129] B. Harink, S. Le Gac, R. Truckenmuller, C. van Blitterswijk, P. Habibovic, Regeneration-on-a-chip? The perspectives on use of microfluidics in regenerative medicine, Lab Chip 13 (2013) 3512–3528.
[130] M.W. Toepke, D.J. Beebe, PDMS absorption of small molecules and consequences in microfluidic applications, Lab Chip 6 (2006) 1484–1486.
[131] K.J. Regehr, M. Domenech, J.T. Koepsel, K.C. Carver, S.J. Ellison-Zelski, W.L. Murphy, L.A. Schuler, E.T. Alarid, D.J. Beebe, Biological implications of polydimethylsiloxane-based microfluidic cell culture, Lab Chip 9 (2009) 2132–2139.
[132] W.P. Daley, S.B. Peters, M. Larsen, Extracellular matrix dynamics in development and regenerative medicine, J. Cell Sci. 121 (2008) 255–264.
[133] S. Jitraruch, A. Dhawan, R.D. Hughes, C. Filippi, D. Soong, C. Philippeos, S.C. Lehec, N. D. Heaton, M.S. Longhi, R.R. Mitry, Alginate microencapsulated hepatocytes optimised for transplantation in acute liver failure, PLoS One 9 (2014), e113609.
[134] T. Maguire, A.E. Davidovich, E.J. Wallenstein, E. Novik, N. Sharma, H. Pedersen, I.P. Androulakis, R. Schloss, M. Yarmush, Control of hepatic differentiation via cellular aggregation in an alginate microenvironment, Biotechnol. Bioeng. 98 (2007) 631–644.
[135] K. Cameron, R. Tan, W. Schmidt-Heck, G. Campos, M.J. Lyall, Y. Wang, B. Lucendo-Villarin, D. Szkolnicka, N. Bates, S.J. Kimber, J.G. Hengstler, P. Godoy, S.J. Forbes, D.C. Hay, Recombinant laminins drive the differentiation and self-organization of hESC-derived hepatocytes, Stem Cell Rep. 5 (2015) 1250–1262.
[136] B. Lucendo-Villarin, H. Rashidi, K. Cameron, D.C. Hay, Pluripotent stem cell derived hepatocytes: using materials to define cellular differentiation and tissue engineering, J. Mater. Chem. B 4 (2016) 3433–3442.
[137] X. Qian, L.G. Villa-Diaz, R. Kumar, J. Lahann, P.H. Krebsbach, Enhancement of the propagation of human embryonic stem cells by modifications in the gel architecture of PMEDSAH polymer coatings, Biomaterials 35 (2014) 9581–9590.
[138] D.T. Chiu, N.L. Jeon, S. Huang, R.S. Kane, C.J. Wargo, I.S. Choi, D.E. Ingber, G.M. Whitesides, Patterned deposition of cells and proteins onto surfaces by using three-dimensional microfluidic systems, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 2408–2413.
[139] B.J. Kane, M.J. Zinner, M.L. Yarmush, M. Toner, Liver-specific functional studies in a microfluidic array of primary mammalian hepatocytes, Anal. Chem. 78 (2006) 4291–4298.
[140] B.M. Baker, B. Trappmann, S.C. Stapleton, E. Toro, C.S. Chen, Microfluidics embedded within extracellular matrix to define vascular architectures and pattern diffusive gradients, Lab Chip 13 (2013) 3246–3252.

[141] M. Raasch, K. Rennert, T. Jahn, S. Peters, T. Henkel, O. Huber, I. Schulz, H. Becker, S. Lorkowski, H. Funke, A. Mosig, Microfluidically supported biochip design for culture of endothelial cell layers with improved perfusion conditions, Biofabrication 7 (2015) 015013.
[142] M. Radisic, W. Deen, R. Langer, G. Vunjak-Novakovic, Mathematical model of oxygen distribution in engineered cardiac tissue with parallel channel array perfused with culture medium containing oxygen carriers, Am. J. Physiol. Heart Circ. Physiol. 288 (2005) H1278–H1289.
[143] N. Li Jeon, H. Baskaran, S.K. Dertinger, G.M. Whitesides, L. Van de Water, M. Toner, Neutrophil chemotaxis in linear and complex gradients of interleukin-8 formed in a microfabricated device, Nat. Biotechnol. 20 (2002) 826–830.
[144] H.J. Kim, D. Huh, G. Hamilton, D.E. Ingber, Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow, Lab Chip 12 (2012) 2165–2174.
[145] S.N. Bhatia, D.E. Ingber, Microfluidic organs-on-chips, Nat. Biotechnol. 32 (2014) 760–772.
[146] D. Huh, G.A. Hamilton, D.E. Ingber, From 3D cell culture to organs-on-chips, Trends Cell Biol. 21 (2011) 745–754.
[147] G.M. Whitesides, The origins and the future of microfluidics, Nature 442 (2006) 368–373.
[148] S.A. Mousavi Shaegh, F. De Ferrari, Y.S. Zhang, M. Nabavinia, N. Binth Mohammad, J. Ryan, A. Pourmand, E. Laukaitis, R. Banan Sadeghian, A. Nadhman, S.R. Shin, A.S. Nezhad, A. Khademhosseini, M.R. Dokmeci, A microfluidic optical platform for real-time monitoring of pH and oxygen in microfluidic bioreactors and organ-on-chip devices, Biomicrofluidics 10 (2016) 044111.
[149] K. Rennert, S. Steinborn, M. Groger, B. Ungerbock, A.M. Jank, J. Ehgartner, S. Nietzsche, J. Dinger, M. Kiehntopf, H. Funke, F.T. Peters, A. Lupp, C. Gartner, T. Mayr, M. Bauer, O. Huber, A.S. Mosig, A microfluidically perfused three dimensional human liver model, Biomaterials 71 (2015) 119–131.
[150] D. Huh, Y.S. Torisawa, G.A. Hamilton, H.J. Kim, D.E. Ingber, Microengineered physiological biomimicry: organs-on-chips, Lab Chip 12 (2012) 2156–2164.
[151] E.A. Corbin, B.R. Dorvel, L.J. Millet, W.P. King, R. Bashir, Micro-patterning of mammalian cells on suspended MEMS resonant sensors for long-term growth measurements, Lab Chip 14 (2014) 1401–1404.
[152] E. Leclerc, K. Kimura, M. Shinohara, M. Danoy, M. Le Gall, T. Kido, A. Miyajima, T. Fujii, Y. Sakai, Comparison of the transcriptomic profile of hepatic human induced pluripotent stem like cells cultured in plates and in a 3D microscale dynamic environment, Genomics 109 (2017) 16–26.
[153] D.E. Kehoe, D. Jing, L.T. Lock, E.S. Tzanakakis, Scalable stirred-suspension bioreactor culture of human pluripotent stem cells, Tissue Eng. Part A 16 (2010) 405–421.
[154] L. Sivertsson, J. Synnergren, J. Jensen, P. Bjorquist, M. Ingelman-Sundberg, Hepatic differentiation and maturation of human embryonic stem cells cultured in a perfused three-dimensional bioreactor, Stem Cells Dev. 22 (2013) 581–594.
[155] T. Miki, A. Ring, J. Gerlach, Hepatic differentiation of human embryonic stem cells is promoted by three-dimensional dynamic perfusion culture conditions, Tissue Eng. Part C Methods 17 (2011) 557–568.
[156] N. Freyer, F. Knospel, N. Strahl, L. Amini, P. Schrade, S. Bachmann, G. Damm, D. Seehofer, F. Jacobs, M. Monshouwer, K. Zeilinger, Hepatic differentiation of human induced pluripotent stem cells in a perfused three-dimensional multicompartment bioreactor, Biores Open Access 5 (2016) 235–248.
[157] K. Zeilinger, T. Schreiter, M. Darnell, T. Soderdahl, M. Lubberstedt, B. Dillner, D. Knobeloch, A.K. Nussler, J.C. Gerlach, T.B. Andersson, Scaling down of a clinical three-dimensional perfusion multicompartment hollow fiber liver bioreactor developed for extracorporeal liver support to an analytical scale device useful for hepatic pharmacological in vitro studies, Tissue Eng. Part C Methods 17 (2011) 549–556.
[158] P.J. Lee, P.J. Hung, L.P. Lee, An artificial liver sinusoid with a microfluidic endothelial-like barrier for primary hepatocyte culture, Biotechnol. Bioeng. 97 (2007) 1340–1346.
[159] S.R. Khetani, S.N. Bhatia, Microscale culture of human liver cells for drug development, Nat. Biotechnol. 26 (2008) 120–126.
[160] C.H. Cho, J. Park, A.W. Tilles, F. Berthiaume, M. Toner, M.L. Yarmush, Layered patterning of hepatocytes in co-culture systems using microfabricated stencils, BioTechniques 48 (2010) 47–52.
[161] S.F. Wong, Y. No da, Y.Y. Choi, D.S. Kim, B.G. Chung, S.H. Lee, Concave microwell based size-controllable hepatosphere as a three-dimensional liver tissue model, Biomaterials 32 (2011) 8087–8096.
[162] S.A. Lee, Y. No da, E. Kang, J. Ju, D.S. Kim, S.H. Lee, Spheroid-based three-dimensional liver-on-a-chip to investigate hepatocyte-hepatic stellate cell interactions and flow effects, Lab Chip 13 (2013) 3529–3537.
[163] A. Shlomai, R.E. Schwartz, V. Ramanan, A. Bhatta, Y.P. de Jong, S.N. Bhatia, C.M. Rice, Modeling host interactions with hepatitis B virus using primary and induced pluripotent stem cell-derived hepatocellular systems, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 12193–12198.
[164] A. Ploss, S.R. Khetani, C.T. Jones, A.J. Syder, K. Trehan, V.A. Gaysinskaya, K. Mu, K. Ritola, C.M. Rice, S.N. Bhatia, Persistent hepatitis C virus infection in microscale primary human hepatocyte cultures, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 3141–3145.
[165] S. March, S. Ng, S. Velmurugan, A. Galstian, J. Shan, D.J. Logan, A.E. Carpenter, D. Thomas, B.K. Sim, M.M. Mota, S.L. Hoffman, S.N. Bhatia, A microscale human liver platform that supports the hepatic stages of Plasmodium falciparum and vivax, Cell Host Microbe 14 (2013) 104–115.
[166] A. Sivaraman, J.K. Leach, S. Townsend, T. Iida, B.J. Hogan, D.B. Stolz, R. Fry, L.D. Samson, S.R. Tannenbaum, L.G. Griffith, A microscale in vitro physiological model of the liver: predictive screens for drug metabolism and enzyme induction, Curr. Drug Metab. 6 (2005) 569–591.
[167] K. Domansky, W. Inman, J. Serdy, A. Dash, M.H. Lim, L.G. Griffith, Perfused multiwell plate for 3D liver tissue engineering, Lab Chip 10 (2010) 51–58.
[168] Y.B. Kang, T.R. Sodunke, J. Lamontagne, J. Cirillo, C. Rajiv, M.J. Bouchard, M. Noh, Liver sinusoid on a chip: long-term layered co-culture of primary rat hepatocytes and endothelial cells in microfluidic platforms, Biotechnol. Bioeng. 112 (2015) 2571–2582.
[169] J. Kasuya, R. Sudo, T. Mitaka, M. Ikeda, K. Tanishita, Spatio-temporal control of hepatic stellate cell-endothelial cell interactions for reconstruction of liver sinusoids in vitro, Tissue Eng. Part A 18 (2012) 1045–1056.
[170] L. Prodanov, R. Jindal, S.S. Bale, M. Hegde, W.J. McCarty, I. Golberg, A. Bhushan, M.L. Yarmush, O.B. Usta, Long-term maintenance of a microfluidic 3D human liver sinusoid, Biotechnol. Bioeng. 113 (2016) 241–246.
[171] Y.S. Weng, S.F. Chang, M.C. Shih, S.H. Tseng, C.H. Lai, Scaffold-free liver-on-A-chip with multiscale organotypic cultures, Adv. Mater. 29 (2017).
[172] P. Chao, T. Maguire, E. Novik, K.C. Cheng, M.L. Yarmush, Evaluation of a microfluidic based cell culture platform with primary human hepatocytes for the prediction of hepatic clearance in human, Biochem. Pharmacol. 78 (2009) 625–632.
[173] E. Novik, T.J. Maguire, P. Chao, K.C. Cheng, M.L. Yarmush, A microfluidic hepatic co-culture platform for cell-based drug metabolism studies, Biochem. Pharmacol. 79 (2010) 1036–1044.
[174] J.Y. Kim, D.A. Fluri, J.M. Kelm, A. Hierlemann, O. Frey, 96-well format-based microfluidic platform for parallel interconnection of multiple multicellular spheroids, J. Lab. Autom. 20 (2015) 274–282.
[175] O. Frey, P.M. Misun, D.A. Fluri, J.G. Hengstler, A. Hierlemann, Reconfigurable microfluidic hanging drop network for multi-tissue interaction and analysis, Nat. Commun. 5 (2014) 4250.
[176] D. Bavli, S. Prill, E. Ezra, G. Levy, M. Cohen, M. Vinken, J. Vanfleteren, M. Jaeger, Y. Nahmias, Real-time monitoring of metabolic function in liver-on-chip microdevices tracks the dynamics of mitochondrial dysfunction, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) E2231–E2240.
[177] R. Gebhardt, M. Matz-Soja, Liver zonation: novel aspects of its regulation and its impact on homeostasis, World J. Gastroenterol. 20 (2014) 8491–8504.
[178] D. Beyoglu, J.R. Idle, The metabolomic window into hepatobiliary disease, J. Hepatol. 59 (2013) 842–858.
[179] R. Conti, E. Mannucci, P. Pessotto, E. Tassoni, P. Carminati, F. Giannessi, A. Arduini, Selective reversible inhibition of liver carnitine palmitoyl-transferase 1 by teglicar reduces gluconeogenesis and improves glucose homeostasis, Diabetes 60 (2011) 644–651.
[180] F.T. Lee-Montiel, S.M. George, A.H. Gough, A.D. Sharma, J. Wu, R. DeBiasio, L.A. Vernetti, D.L. Taylor, Control of oxygen tension recapitulates zone-specific functions in human liver microphysiology systems, Exp. Biol. Med. (Maywood) 242 (2017) 1617–1632.
[181] W.J. McCarty, O.B. Usta, M.L. Yarmush, A microfabricated platform for generating physiologically-relevant hepatocyte zonation, Sci. Rep. 6 (2016) 26868.
[182] M. Groger, K. Rennert, B. Giszas, E. Weiss, J. Dinger, H. Funke, M. Kiehntopf, F.T. Peters, A. Lupp, M. Bauer, R.A. Claus, O. Huber, A.S. Mosig, Monocyte-induced recovery of inflammation-associated hepatocellular dysfunction in a biochip-based human liver model, Sci. Rep. 6 (2016) 21868.
[183] A.J. van Hoek, N. Andrews, P.A. Waight, J. Stowe, P. Gates, R. George, E. Miller, The effect of underlying clinical conditions on the risk of developing invasive pneumococcal disease in England, J. Inf. Secur. 65 (2012) 17–24.
[184] T. Bruns, H.W. Zimmermann, A. Stallmach, Risk factors and outcome of bacterial infections in cirrhosis, World J. Gastroenterol. 20 (2014) 2542–2554.
[185] M.W. Whitehead, I. Hainsworth, J.G. Kingham, The causes of obvious jaundice in south West Wales: perceptions versus reality, Gut 48 (2001) 409–413.
[186] P. Recknagel, F.A. Gonnert, M. Westermann, S. Lambeck, A. Lupp, A. Rudiger, A. Dyson, J.E. Carre, A. Kortgen, C. Krafft, J. Popp, C. Sponholz, V. Fuhrmann, I. Hilger, R.A. Claus, N.C. Riedemann, R. Wetzker, M. Singer, M. Trauner, M. Bauer, Liver dysfunction and phosphatidylinositol-3-kinase signalling in early sepsis: experimental studies in rodent models of peritonitis, PLoS Med. 9 (2012), e1001338.
[187] E. Hirsch, V.L. Katanaev, C. Garlanda, O. Azzolino, L. Pirola, L. Silengo, S. Sozzani, A. Mantovani, F. Altruda, M.P. Wymann, Central role for G protein-coupled phosphoinositide 3-kinase gamma in inflammation, Science 287 (2000) 1049–1053.
[188] A.T. Press, A. Traeger, C. Pietsch, A. Mosig, M. Wagner, M.G. Clemens, N. Jbeily, N. Koch, M. Gottschaldt, N. Beziere, V. Ermolayev, V. Ntziachristos, J. Popp, M.M. Kessels, B. Qualmann, U.S. Schubert, M. Bauer, Cell type-specific delivery of short interfering RNAs by dye-functionalised theranostic nanoparticles, Nat. Commun. 5 (2014) 5565.
[189] A.T. Press, A. Ramoji, V.L. M, A.C. R, J. Hoff, M. Butans, C. Rössel, C. Pietsch, U. Neugebauer, F.H. Schacher, M. Bauer, Cargo–carrier interactions significantly contribute to micellar conformation and biodistribution, NPG Asia Mater. 9 (2017).
[190] A.C. Rinkenauer, A.T. Press, M. Raasch, C. Pietsch, S. Schweizer, S. Schworer, K.L. Rudolph, A. Mosig, M. Bauer, A. Traeger, U.S. Schubert, Comparison of the uptake of methacrylate-based nanoparticles in static and dynamic in vitro systems as well as in vivo, J. Control. Release 216 (2015) 158–168.
[191] R.E. Schwartz, K. Trehan, L. Andrus, T.P. Sheahan, A. Ploss, S.A. Duncan, C.M. Rice, S. N. Bhatia, Modeling hepatitis C virus infection using human induced pluripotent stem cells, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 2544–2548.
[192] K. Si-Tayeb, J.C. Duclos-Vallee, M.A. Petit, Hepatocyte-like cells differentiated from human induced pluripotent stem cells (iHLCs) are permissive to hepatitis C virus (HCV) infection: HCV study gets personal, J. Hepatol. 57 (2012) 689–691.
[193] K. Sa-Ngiamsuntorn, A. Wongkajornsilp, P. Phanthong, S. Borwornpinyo, N. Kitiyanant, W. Chantratita, S. Hongeng, A robust model of natural hepatitis C infection using hepatocyte-like cells derived from human induced pluripotent stem cells as a long-term host, Virol. J. 13 (2016) 59.
[194] S. Ng, R.E. Schwartz, S. March, A. Galstian, N. Gural, J. Shan, M. Prabhu, M.M. Mota, S.N. Bhatia, Human iPSC-derived hepatocyte-like cells support Plasmodium liver-stage infection in vitro, Stem Cell Rep. 4 (2015) 348–359.

[195] U. Protzer, M.K. Maini, P.A. Knolle, Living in the liver: hepatic infections, Nat. Rev. Immunol. 12 (2012) 201–213.
[196] M. van Egmond, E. van Garderen, A.B. van Spriel, C.A. Damen, E.S. van Amersfoort, G. van Zandbergen, J. van Hattum, J. Kuiper, J.G. van de Winkel, FcalphaRI-positive liver Kupffer cells: reappraisal of the function of immunoglobulin A in immunity, Nat. Med. 6 (2000) 680–685.
[197] Y. Debing, D. Moradpour, J. Neyts, J. Gouttenoire, Update on hepatitis E virology: implications for clinical practice, J. Hepatol. 65 (2016) 200–212.
[198] H. Yan, Y. Liu, J. Sui, W. Li, NTCP opens the door for hepatitis B virus infection, Antivir. Res. 121 (2015) 24–30.
[199] A. Schulze, P. Gripon, S. Urban, Hepatitis B virus infection initiates with a large surface protein-dependent binding to heparan sulfate proteoglycans, Hepatology 46 (2007) 1759–1768.
[200] A.R. Jilbert, D.S. Miller, C.A. Scougall, H. Turnbull, C.J. Burrell, Kinetics of duck hepatitis B virus infection following low dose virus inoculation: one virus DNA genome is infectious in neonatal ducks, Virology 226 (1996) 338–345.
[201] S. Asabe, S.F. Wieland, P.K. Chattopadhyay, M. Roederer, R.E. Engle, R.H. Purcell, F.V. Chisari, The size of the viral inoculum contributes to the outcome of hepatitis B virus infection, J. Virol. 83 (2009) 9652–9662.
[202] P. Gripon, S. Rumin, S. Urban, J. Le Seyec, D. Glaise, I. Cannie, C. Guyomard, J. Lucas, C. Trepo, C. Guguen-Guillouzo, Infection of a human hepatoma cell line by hepatitis B virus, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 15655–15660.
[203] M.P. Manns, M. Buti, E. Gane, J.M. Pawlotsky, H. Razavi, N. Terrault, Z. Younossi, Hepatitis C virus infection, Nat. Rev. Dis. Primers 3 (2017) 17006.
[204] I. Schietroma, G.C. Scheri, C. Pinacchio, M. Statzu, A. Petruzziello, V. Vullo, Hepatitis C virus and hepatocellular carcinoma: pathogenetic mechanisms and impact of direct-acting antivirals, Open Virol J. 12 (2018) 16–25.
[205] P. Pileri, Y. Uematsu, S. Campagnoli, G. Galli, F. Falugi, R. Petracca, A.J. Weiner, M. Houghton, D. Rosa, G. Grandi, S. Abrignani, Binding of hepatitis C virus to CD81, Science 282 (1998) 938–941.
[206] V. Agnello, G. Abel, M. Elfahal, G.B. Knight, Q.X. Zhang, Hepatitis C virus and other flaviviridae viruses enter cells via low density lipoprotein receptor, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 12766–12771.
[207] E. Scarselli, H. Ansuini, R. Cerino, R.M. Roccasecca, S. Acali, G. Filocamo, C. Traboni, A. Nicosia, R. Cortese, A. Vitelli, The human scavenger receptor class B type I is a novel candidate receptor for the hepatitis C virus, EMBO J. 21 (2002) 5017–5025.
[208] M.J. Evans, T. von Hahn, D.M. Tscherne, A.J. Syder, M. Panis, B. Wolk, T. Hatziioannou, J.A. McKeating, P.D. Bieniasz, C.M. Rice, Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry, Nature 446 (2007) 801–805.
[209] A. Ploss, M.J. Evans, V.A. Gaysinskaya, M. Panis, H. You, Y.P. de Jong, C.M. Rice, Human occludin is a hepatitis C virus entry factor required for infection of mouse cells, Nature 457 (2009) 882–886.
[210] J. Lupberger, M.B. Zeisel, F. Xiao, C. Thumann, I. Fofana, L. Zona, C. Davis, C.J. Mee, M. Turek, S. Gorke, C. Royer, B. Fischer, M.N. Zahid, D. Lavillette, J. Fresquet, F.L. Cosset, S.M. Rothenberg, T. Pietschmann, A.H. Patel, P. Pessaux, M. Doffoel, W. Raffelsberger, O. Poch, J.A. McKeating, L. Brino, T.F. Baumert, EGFR and EphA2 are host factors for hepatitis C virus entry and possible targets for antiviral therapy, Nat. Med. 17 (2011) 589–595.
[211] R. Bartenschlager, F. Penin, V. Lohmann, P. Andre, Assembly of infectious hepatitis C virus particles, Trends Microbiol. 19 (2011) 95–103.
[212] T. Yoshida, K. Takayama, M. Kondoh, F. Sakurai, H. Tani, N. Sakamoto, Y. Matsuura, H. Mizuguchi, K. Yagi, Use of human hepatocyte-like cells derived from induced pluripotent stem cells as a model for hepatocytes in hepatitis C virus infection, Biochem. Biophys. Res. Commun. 416 (2011) 119–124.
[213] X. Wu, J.M. Robotham, E. Lee, S. Dalton, N.M. Kneteman, D.M. Gilbert, H. Tang, Productive hepatitis C virus infection of stem cell-derived hepatocytes reveals a critical transition to viral permissiveness during differentiation, PLoS Pathog. 8 (2012), e1002617.
[214] G.E. Thwaites, J.D. Edgeworth, E. Gkrania-Klotsas, A. Kirby, R. Tilley, M.E. Torok, S. Walker, H.F. Wertheim, P. Wilson, M.J. Llewelyn, U.K.C.I.R. Group, Clinical management of *Staphylococcus aureus* bacteraemia, Lancet Infect. Dis. 11 (2011) 208–222.
[215] E. Kolaczkowska, C.N. Jenne, B.G. Surewaard, A. Thanabalasuriar, W.Y. Lee, M.J. Sanz, K. Mowen, G. Opdenakker, P. Kubes, Molecular mechanisms of NET formation and degradation revealed by intravital imaging in the liver vasculature, Nat. Commun. 6 (2015) 6673.
[216] P.A. Knolle, G. Gerken, Local control of the immune response in the liver, Immunol. Rev. 174 (2000) 21–34.
[217] H.M. Cantor, A.E. Dumont, Hepatic suppression of sensitization to antigen absorbed into the portal system, Nature 215 (1967) 744–745.
[218] M.P. Callery, T. Kamei, M.W. Flye, Kupffer cell blockade inhibits induction of tolerance by the portal venous route, Transplantation 47 (1989) 1092–1094.
[219] F. Sierro, M. Evrard, S. Rizzetto, M. Melino, A.J. Mitchell, M. Florido, L. Beattie, S.B. Walters, S.S. Tay, B. Lu, L.E. Holz, B. Roediger, Y.C. Wong, A. Warren, W. Ritchie, C. Mcguffog, W. Weninger, D.G. Le Couteur, F. Ginhoux, W.J. Britton, W.R. Heath, B. M. Saunders, G.W. Mccaughan, F. Luciani, K.P.A. MacDonald, L.G. Ng, D.G. Bowen, P. Bertolino, A liver capsular network of monocyte-derived macrophages restricts hepatic dissemination of intraperitoneal bacteria by neutrophil recruitment, Immunity 47 (2017) 374–388 (e376).
[220] A. Popov, Z. Abdullah, C. Wickenhauser, T. Saric, J. Driesen, F.G. Hanisch, E. Domann, E.L. Raven, O. Dehus, C. Hermann, D. Eggle, S. Debey, T. Chakraborty, M. Kronke, O. Utermohlen, J.L. Schultze, Indoleamine 2,3-dioxygenase-expressing dendritic cells form suppurative granulomas following Listeria monocytogenes infection, J. Clin. Invest. 116 (2006) 3160–3170.
[221] J.G. Egen, A.G. Rothfuchs, C.G. Feng, N. Winter, A. Sher, R.N. Germain, Macrophage and T cell dynamics during the development and disintegration of mycobacterial granulomas, Immunity 28 (2008) 271–284.
[222] H.E. Volkman, T.C. Pozos, J. Zheng, J.M. Davis, J.F. Rawls, L. Ramakrishnan, Tuberculous granuloma induction via interaction of a bacterial secreted protein with host epithelium, Science 327 (2010) 466–469.
[223] J.L. Taylor, J.M. Hattle, S.A. Dreitz, J.M. Troudt, L.S. Izzo, R.J. Basaraba, I.M. Orme, L.M. Matrisian, A.A. Izzo, Role for matrix metalloproteinase 9 in granuloma formation during pulmonary *Mycobacterium* tuberculosis infection, Infect. Immun. 74 (2006) 6135–6144.
[224] M.K. McLendon, M.A. Apicella, L.A. Allen, Francisella tularensis: taxonomy, genetics, and immunopathogenesis of a potential agent of biowarfare, Annu. Rev. Microbiol. 60 (2006) 167–185.
[225] L. del Barrio, M. Sahoo, L. Lantier, J.M. Reynolds, I. Ceballos-Olvera, F. Re, Production of anti-LPS IgM by B1a B cells depends on IL-1beta and is protective against lung infection with *Francisella tularensis* LVS, PLoS Pathog. 11 (2015), e1004706.
[226] D. Chiavolini, J. Alroy, C.A. King, P. Jorth, S. Weir, G. Madico, J.R. Murphy, L.M. Wetzler, Identification of immunologic and pathologic parameters of death versus survival in respiratory tularemia, Infect. Immun. 76 (2008) 486–496.
[227] K. Rennert, P. Otto, H. Funke, O. Huber, H. Tomaso, A.S. Mosig, A human macrophage-hepatocyte co-culture model for comparative studies of infection and replication of *Francisella tularensis* LVS strain and subspecies holarctica and mediasiatica, BMC Microbiol. 16 (2016) 2.
[228] G. Caggiano, C. Coretti, N. Bartolomeo, G. Lovero, O. De Giglio, M.T. Montagna, Candida bloodstream infections in Italy: changing epidemiology during 16 years of surveillance, Biomed. Res. Int. 2015 (2015) 256580.
[229] M.S. Lionakis, J.K. Lim, C.C. Lee, P.M. Murphy, Organ-specific innate immune responses in a mouse model of invasive candidiasis, J. Innate Immun. 3 (2011) 180–199.
[230] X. Tang, H. Zhu, L. Sun, W. Hou, S. Cai, R. Zhang, F. Liu, Enhanced antifungal effects of amphotericin B-TPGS-b-(PCL-ran-PGA) nanoparticles in vitro and in vivo, Int. J. Nanomedicine 9 (2014) 5403–5413.
[231] J.P. Wong, H. Yang, K.L. Blasetti, G. Schnell, J. Conley, L.N. Schofield, Liposome delivery of ciprofloxacin against intracellular *Francisella tularensis* infection, J. Control. Release 92 (2003) 265–273.
[232] Y. Pei, M.F. Mohamed, M.N. Seleem, Y. Yeo, Particle engineering for intracellular delivery of vancomycin to methicillin-resistant *Staphylococcus aureus* (MRSA)-infected macrophages, J. Control. Release 267 (2017) 133–143.
[233] M.M. Mota, J.C. Hafalla, A. Rodriguez, Migration through host cells activates *Plasmodium sporozoites* for infection, Nat. Med. 8 (2002) 1318–1322.
[234] T. Ishino, K. Yano, Y. Chinzei, M. Yuda, Cell-passage activity is required for the malarial parasite to cross the liver sinusoidal cell layer, PLoS Biol. 2 (2004), E4.
[235] K. Baer, M. Roosevelt, A.B. Clarkson Jr., N. van Rooijen, T. Schnieder, U. Frevert, Kupffer cells are obligatory for *Plasmodium yoelii* sporozoite infection of the liver, Cell. Microbiol. 9 (2007) 397–412.
[236] G. Pradel, U. Frevert, Malaria sporozoites actively enter and pass through rat Kupffer cells prior to hepatocyte invasion, Hepatology 33 (2001) 1154–1165.
[237] M.M. Mota, G. Pradel, J.P. Vanderberg, J.C. Hafalla, U. Frevert, R.S. Nussenzweig, V. Nussenzweig, A. Rodriguez, Migration of *Plasmodium sporozoites* through cells before infection, Science 291 (2001) 141–144.
[238] A. Sturm, R. Amino, C. van de Sand, T. Regen, S. Retzlaff, A. Rennenberg, A. Krueger, J.M. Pollok, R. Menard, V.T. Heussler, Manipulation of host hepatocytes by the malaria parasite for delivery into liver sinusoids, Science 313 (2006) 1287–1290.
[239] G. Vunjak-Novakovic, S. Bhatia, C. Chen, K. Hirschi, HeLiVa platform: integrated heart-liver-vascular systems for drug testing in human health and disease, Stem Cell Res Ther 1 (4 Suppl) (2013) S8.
[240] J. Fernandez, F. Bert, M.H. Nicolas-Chanoine, The challenges of multi-drug-resistance in hepatology, J. Hepatol. 65 (2016) 1043–1054.
[241] M. Vouga, G. Greub, Emerging bacterial pathogens: the past and beyond, Clin. Microbiol. Infect. 22 (2016) 12–21.
[242] L.K. Siu, K.M. Yeh, J.C. Lin, C.P. Fung, F.Y. Chang, *Klebsiella pneumoniae* liver abscess: a new invasive syndrome, Lancet Infect. Dis. 12 (2012) 881–887.
[243] K. Viravaidya, A. Sin, M.L. Shuler, Development of a microscale cell culture analog to probe naphthalene toxicity, Biotechnol. Prog. 20 (2004) 316–323.
[244] M.B. Esch, G.J. Mahler, T. Stokol, M.L. Shuler, Body-on-a-chip simulation with gastrointestinal tract and liver tissues suggests that ingested nanoparticles have the potential to cause liver injury, Lab Chip 14 (2014) 3081–3092.
[245] T. Bricks, P. Paullier, A. Legendre, M.J. Fleury, P. Zeller, F. Merlier, P.M. Anton, E. Leclerc, Development of a new microfluidic platform integrating co-cultures of intestinal and liver cell lines, Toxicol. in Vitro 28 (2014) 885–895.
[246] L. Choucha-Snouber, C. Aninat, L. Grsicom, G. Madalinski, C. Brochot, P.E. Poleni, F. Razan, C.G. Guillouzo, C. Legallais, A. Corlu, E. Leclerc, Investigation of ifosfamide nephrotoxicity induced in a liver-kidney co-culture biochip, Biotechnol. Bioeng. 110 (2013) 597–608.
[247] E.M. Materne, A.P. Ramme, A.P. Terrasso, M. Serra, P.M. Alves, C. Brito, D.A. Sakharov, A.G. Tonevitsky, R. Lauster, U. Marx, A multi-organ chip co-culture of neurospheres and liver equivalents for long-term substance testing, J. Biotechnol. 205 (2015) 36–46.
[248] I. Wagner, E.M. Materne, S. Brincker, U. Sussbier, C. Fradrich, M. Busek, F. Sonntag, D.A. Sakharov, E.V. Trushkin, A.G. Tonevitsky, R. Lauster, U. Marx, A dynamic multi-organ-chip for long-term cultivation and substance testing proven by 3D human liver and skin tissue co-culture, Lab Chip 13 (2013) 3538–3547.
[249] M.B. Bracken, Why animal studies are often poor predictors of human reactions to exposure, J. R. Soc. Med. 102 (2009) 120–122.