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Bioengineering of the liver

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INTRODUCTION

The liver is an extremely complicated organ regulating the crucial metabolic processes and immune homeostasis in the human body. It performs various functions, such as carbohydrate, lipid, and amino acid metabolism, ammonia clearance, urea synthesis, albumin and bile acid synthesis, xenobiotic metabolism, and inflammatory response.[1] Various pathogenic factors, including alcohol abuse, viral infection, and autoimmune or metabolic disorders, promote functional disorders of the liver, inducing acute or chronic inflammation, fibrosis, cirrhosis, and even tumorigenesis. Meanwhile, the liver is located in a complicated mechanical or physical microenvironment that is critical for maintaining physiological homeostasis, possessing mechanotransductive responses of various hepatic cells.[2] Emerging bioengineering technologies enable efficient assessment and tests of liver physiopathology, covering the fields of microfluidics, biomaterials, tissue engineering and bioprinting, gene screening and genotyping, biomechanics and mechanobiology, and others.[3,4] The latest advances addressed include organ-on-chips, organoids, gene sequencing, drug release profiling, and cytotoxicity screening, providing an overview from basic research to translation into practice and describing the most exciting challenges and opportunities that multidisciplinary approaches can provide to the field.

APL Bioengineering pays much attention to inviting special issues and collections that aim to highlight major medical and health challenges and exemplify innovative contributions to address the challenges. In this collection of papers, we underline the contributions recently published in *APL Bioengineering*, aimed at further understanding hepatic function and homeostasis of human and animal subjects, how human physiology and pathology can be reasonably mimicked with *in vitro* liver tissue or organ models, and how drug and therapeutic discovery with engineered liver platforms can address liver diseases. This collection represents a broad range of contributions by leading bioengineering experts in the areas of hepatic physiopathology, animal models of liver cancer, liver-on-a-chip, or Multi-Organs-on-Chip (MOoC), and drug development and tests of hepatic diseases.

This "*Bioengineering of the Liver*" collection includes six papers. The first three papers deal with hepatic physiopathology and related innovative techniques, focusing on highlighting the liver-on-a-chip models to improve the physiopathological relevance of liver tissues and diseases,[5] deciphering the advantages of microfluidic cocultures of human-induced pluripotent stem cell (hiPSC)-derived liver sinusoidal endothelial cells (LSECs) and hepatocytes-like cells (HLCs) in presenting typical hepatic functions,[6] and discussing the benefits of emerging microfluidic-based culture approaches in hepatocyte functional maintenance.[7] These papers offer insights into liver function and homeostasis based on *in vitro* cell models. The other three papers summarize the latest advances in immunotherapies efficacy assessments and drug safety tests by comparing the efficacy of two- (2D) and three-dimensional (3D) *in vitro* cancer models in replicating the hepatocellular carcinoma microenvironmental characteristics and in investigating possible immunotherapy limiting factors,[8] showcasing the impacts of engineered liver platforms on drug development,[9] and specifying the off-target effects of drug safety testing via liver–heart organ-on-chip platform.[10] These papers shed light on a drug test and delivery for the liver from the liver-on-a-chip viewpoint (Fig. 1).

PHYSIOPATHOLOGY/TECHNOLOGY OF THE LIVER

The liver is composed of numerous elementary lobules with radially distributed sinusoids. It has been known that normal liver functions are governed by liver 3D anatomical structure, cell composition, biochemical factors, and biomechanical cues and that liver pathology is associated with these multiple factors.[11,12] Therefore, mimicking *in vivo* physiological relevance using *in vitro* liver culture systems is determined not only by the phenotype and function of hepatocytes and other non-parenchymal cells but also by the appropriate regulation of those biochemical and biomechanical factors.[13] Technically,

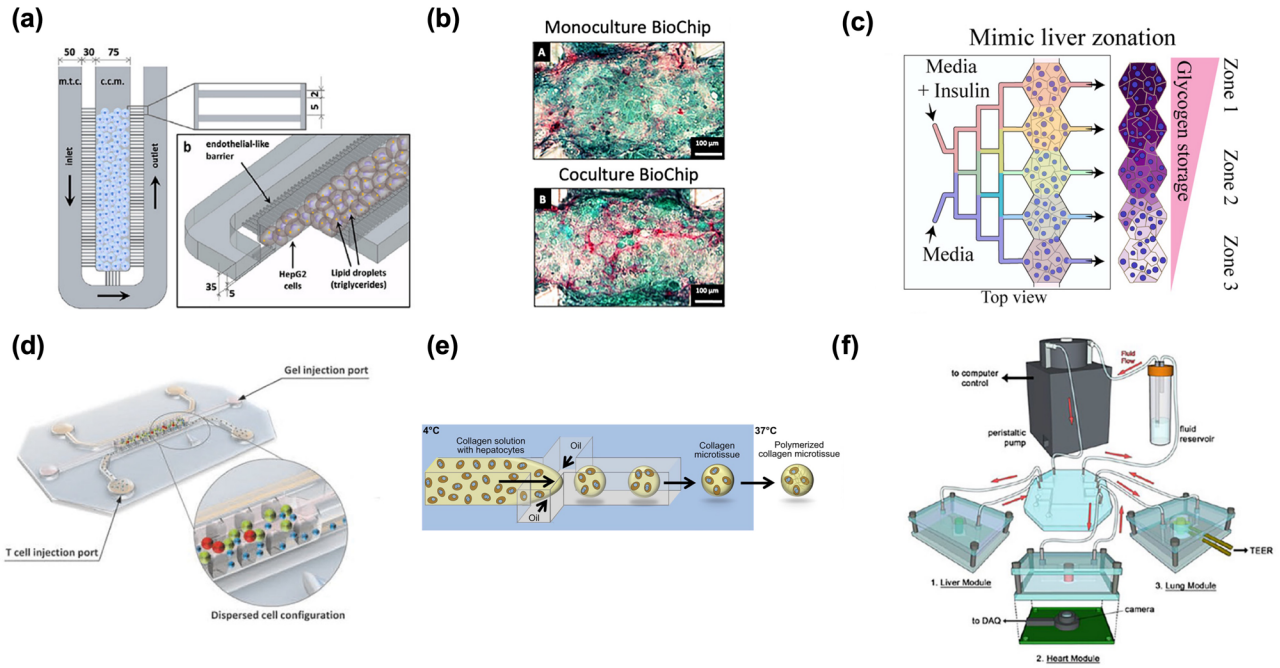

FIG. 1. Exemplified microtechnology-based cell culture models and microfluidic devices for liver physiology and liver diseases. (a) A liver organoid-on-a-chip system aimed at mimicking liver function and pathophysiology.[14] (b) Sirius red and fast green staining of monoculture and coculture biochips from the article of Danoy *et al.*[6] (c) Liver zonation recreated in a microfluidic device from the review of de Hoyos-Vega *et al.*[7] (d) A 3D vascularized tumor model for cancer-specific characterization and drug dissemination from the review of Lam *et al.*[8] (e) A high-throughput droplet microfluidic device for the generation of 3D liver microtissues from the review of Monckton *et al.*[9] (f) Conceptual design of a liver–heart organoids-on-chip system.[17] (a) Reproduced with permission from Gori *et al.*, PLoS One **11**, e0159729 (2016). Copyright 2016 Authors, licensed under a Creative Commons Attribution (CC BY) license;[14] (b) reproduced with permission from Danoy *et al.*, APL Bioeng. **5**, 026104 (2021). Copyright 2021 AIP Publishing;[6] (c) reproduced with permission from de Hoyos-Vega *et al.*, APL Bioeng. **5**, 041504 (2021). Copyright 2021 AIP Publishing;[7] (d) reproduced with permission from Pavesi *et al.*, JCI Insight **2**(12), e89762 (2017). Copyright 2017 Authors, licensed under a Creative Commons Attribution (CC BY) license;[15] (e) reproduced with permission from Kukla *et al.*, Gene Expression **20**(1), 1 (2020). Copyright 2020 Authors, licensed under the Creative Commons CC BY-NC-ND license;[16] (f) reproduced with permission from Skardal *et al.*, Sci. Rep. **7**, 8837 (2017). Copyright 2017 Authors, licensed under a Creative Commons Attribution (CC BY) license.[17]

the latest microtechnology-based microfluidic systems enable us to integrate these complex regulating factors into a single device, attempting to recapitulate their physiopathological relevance to the liver.

Along this line, Lee *et al.*[5] first summarize the recent progress on liver-on-a-chip based on microtechnology that integrates cell culture models and microfluidics. Their review provides the state-of-the-art *in vitro* liver models to mimic closely the *in vivo* hepatic microenvironment and liver diseases, including cell-to-cell and cell-to-extracellular matrix (ECM) interactions, shear flow, and other mechanical stimuli, and concentration gradient of oxygen and signaling molecules [see, e.g., Fig. 1(a)[14]]. The authors specify the impacts of shear stress and endothelial barriers on these *in vitro* models and the importance of 3D cell clusters and disease models to mimic liver physiopathology. They also highlight the MOoCs such as gut–liver models that integrate liver models with interstitial models to recapitulate the gut–liver crosstalk and the chip-based liver disease models used to better understand the mechanisms of disease development and demonstrate the efficacy of drugs. Future perspectives for the liver-on-a-chip models and the challenges of developing a better *in vitro* model are proposed in human-originated cell source, biomechanical cues, immune homeostasis, and real-time monitoring of functional maintenance, underlining how these microtechnology-based *in vitro* models can improve the physiological relevance of hepatic cell behaviors and elaborate the pathology of liver diseases.

However, the efficiency of liver-on-a-chips and MOoCs are strongly dependent on the cell sources and the related culture assays. The shortage of functional hepatocytes hampers the application of these *in vitro* models. In their research article, Danoy *et al.*[6] investigate the hepatic development using the system of hiPSCs-derived LSECs cocultured with HLCs in a fluidic microenvironment [see, e.g., Fig. 1(b)[6]]. They demonstrate that these cocultures with these two cell types inoculated in microfluidic biochips present, compared to the monocultures of HLCs alone, the higher albumin production and CYP450 inducibility with tubular-like LSECs structures, positive endothelial marker PECAM1, as well as highly expressed advanced endothelial hepatic marker Stabilin-2. Even without a marked difference between the transcriptomic profiles of both culture conditions, different upstream regulators are highlighted from comparisons between cocultures (SP1, EBF1, and GATA3) and monocultures (PML, MECP2, and NRF1). Meanwhile, the multi-omics analysis including proteomics and metabolomics, in the cocultures, indicates the activation of signaling related to hepatic maturation, angiogenesis, and tissue repair and the reduction of inflammatory signaling via lowered activation of NFκB and decreased production of tissue injury-related

cytokines. They highlight the potential impacts of these culture systems on the processes of hepatic differentiation and regulation of the inflammatory phenomena.

Not only microtechnology-based culture systems and cell sources are key to constructing the *in vitro* cell models, but hepatic function and phenotypic monitoring of composing cell types are also critical to applying these models. In their review, de Hoyos-Vega *et al.*[7] summarize the *in vitro* hepatocyte cultures with their *in vivo* hepatic phenotype and function. They highlight essential functions of the liver cells and available cell sources for *in vitro* models along with traditional methods for hepatocytes cultivation. The liver possesses various functions including glucose and lipid metabolism, bile and urea production, cytochrome P450- and other enzymes-participating detoxification, and plasma proteins production and secretion. While multiple cell sources of primary hepatocytes, cells from chimeric mice with humanized livers, immortalized cell lines, or pluripotent stem cells are used in *in vitro* culture models, the conventional culture methods mainly aim to extend hepatocyte functional maintenance *in vitro*, using different culture systems such as hepatocytes in ECM gels and other 3D cultures, random or micropatterned cocultures of hepatocytes and non-parenchymal cells, spheroid cultures of hepatocytes, or cultivation of intact liver tissue via precision-cut liver slices. They also discuss hepatocyte cultures in microfluidic devices and the integration of bioanalytical tools into such microfluidic cultures. The advantages of microfluidic hepatocyte cultures are underlined from several aspects of small volume effect, ECM gels incorporation, liver zonation, stem cell hepatic differentiation, multi-type cell coculture, and multi-organs-on-chip, or drug hepatotoxicity prediction [see, e.g., Fig. 1(c)]. Multiple bioanalytical tools such as enzymatic assays, immunoassays, or electrochemical biosensors are able to be/can be coupled to a microfluidic device for online detection of hepatic biomarkers.

DRUG EFFICACY AND TOXICITY IN THE LIVER

The availability of platforms capable of replicating liver tissue response is fundamental to testing the effects of advanced therapies with high-throughput and robust tools. The systemic treatment is the elective therapeutical choice for patients diagnosed at the earlier stage of liver tumor progression, but it is poorly effective at the advanced stage. Immunotherapies and adoptive cell therapies (ACTs) are alternative therapeutical approaches under investigation; they have preliminarily demonstrated the potentiality of the immune system in contrasting tumor cells. In particular, ACTs introduce anti-tumor immune cells into the patient rather than relying on the patient's endogenous immune cells; however, preliminary evidence show that their action can be hampered by liver tumor environment according to mechanisms that are not understood yet. Microfluidic platforms have also another emerging application when dealing with liver physiopathology and drugs; adverse effects of drugs on the liver are responsible for 25% of drug withdrawals from the market, which implies elevated societal and economic impacts. The reason is that the liver is highly sensitive to drug toxicity, due to its key role in the metabolic pathways. Microfluidic platform, by being capable of hosting different cell types and applying gradients of soluble factors with unprecedented precision, are unique tools for this kind of experiment.

In this scenario, the review of Lam *et al.*[8] focuses on the most frequent liver cancer, hepatocellular carcinoma, and compares the efficacy of 2D and 3D models in investigating the tumor environment effect on the efficacy of advanced therapies for liver cancer treatment. In the first section of their work, they introduce ACTs, consisting in isolating immune cells from the patient and altering their genetic profile before introducing them back into the patient. In the second section, the effect of the modification of the tumor microenvironment on the onset of an immunosuppressive environment is analyzed, in terms of imbalance of pro-and anti-inflammatory cytokines and cell types presence of inflammatory mediators, abnormal angiogenesis, and tissue remodeling. In the last section. of their work, Lam and colleagues present evidence of the superior performance of microfluidic 3D technology to mimic the physiological conditions of the tumor and to analyze the spatiotemporal relationship between cancer, stroma, and native or modified immune cells in liver cancers [see, e.g., Fig. 1(d)[15]].

The other two works focus on a drug-induced liver injury that is known as a leading cause of drug attrition. While primary human liver cells are ideal for fabricating such models, they rapidly lose their phenotypic functions within conventional 2D *in vitro* models, calling for advanced 3D microfluidic platforms. Monckton and colleagues[9] provide an exhaustive panorama of already engineered human liver models that are commercially available [see, e.g., Fig. 1(e)[16]]. The list includes micropatterned cocultures, spheroids, organoids, bioprinted tissues, and microfluidic devices. Their review well exemplifies how greater levels of realism can be included, moving from micropatterned cocultures to microfluidics platforms, in terms of fluid shear stress, factor gradients, inter-tissue crosstalk, and angiogenesis, where the price to be paid is the increase in costs and protocol complexity. The review also includes a window on the technology of the near future, represented by the emerging body-on-a-chip approach, which combines different organs thus allowing to evaluate the crosstalk between different tissues. This field, still in its infancy, needs further refinement, validation, and standardization but its impact is potentially a breakthrough for the advancement of the drug discovery industry.

This is the topic of the sixth article of the collection that focuses on the interaction between liver and heart. Liver and heart toxicity are the two principal causes of drug failures and their fate is strictly interconnected. The paper by Ferrari and Rasponi[10] offers an overview of the most recently published papers presenting microfluidic platforms for the study of the liver, the heart, and their interaction [see, e.g., Fig. 1(f)[17]]. Indeed, while the liver and heart have been studied separately for several years, MOoCs provide the unprecedented opportunity of studying drug-related effects at the tissue level on several organs, simultaneously and potentially allowing to mimic the absorption, distribution, metabolism, and elimination process of drugs in a way that closely mimics what occurs in the human body.

CONCLUSIONS

Microfluidics technology represents a powerful tool for the study of cancer biology and immune diseases and drug toxicity; it has the potential for scaling-up and high-throughput analysis, speeding up our knowledge of molecular mechanisms of liver diseases and drug development and cell therapy discoveries. In the near future, these *in vitro* models could enable patient clustering and the development of personalized therapies by including cells obtained from biopsies or with stem cell harvesting approaches.

In line with *APL Bioengineering*'s vision, this collection of papers is a clear demonstration that a proper combination of an engineering mindset combined with in-depth biological and physiological

understanding for liver physiopathology can provide unprecedented opportunities, in terms of therapy design, personalized approaches, and technologies that, in this case, potentially translate into an unprecedented opportunity for Pharma and Biotech industries in reducing costs by lessening animal usage and providing patients with safer and better-targeted drugs.

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