Advanced Drug Delivery Reviews 140 (2019) 101–128

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

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

Stem-cell based organ-on-a-chip models for diabetes research

Julia Rogal [a,b,1], Aline Zbinden [a,1], Katja Schenke-Layland [a,c,d,*,2], Peter Loskill [a,b,2]

[a] Department of Women's Health, Research Institute for Women's Health, Eberhard Karls University, Silcherstrasse 7/1, 72076 Tübingen, Germany
[b] Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB, Nobelstrasse 12, 70569 Stuttgart, Germany
[c] The Natural and Medical Sciences Institute (NMI) at the University of Tübingen, Markwiesenstr. 55, 72770 Reutlingen, Germany
[d] Department of Medicine/Cardiology, Cardiovascular Research Laboratories, David Geffen School of Medicine at UCLA, 675 Charles E. Young Drive South, MRL 3645, Los Angeles, CA, USA

ARTICLE INFO

Article history:
Received 4 April 2018
Received in revised form 10 September 2018
Accepted 19 October 2018
Available online 22 October 2018

Keywords:
Type 1 diabetes
Type 2 diabetes
HiPSCs
Human in vitro model
Organ-on-a-chip
Multi-organ chips
Microfluidics

ABSTRACT

Diabetes mellitus (DM) ranks among the severest global health concerns of the 21st century. It encompasses a group of chronic disorders characterized by a dysregulated glucose metabolism, which arises as a consequence of progressive autoimmune destruction of pancreatic beta-cells (type 1 DM), or as a result of beta-cell dysfunction combined with systemic insulin resistance (type 2 DM). Human cohort studies have provided evidence of genetic and environmental contributions to DM; yet, these studies are mostly restricted to investigating statistical correlations between DM and certain risk factors. Mechanistic studies, on the other hand, aimed at re-creating the clinical picture of human DM in animal models. A translation to human biology is, however, often inadequate owing to significant differences between animal and human physiology, including the species-specific glucose regulation. Thus, there is an urgent need for the development of advanced human in vitro models with the potential to identify novel treatment options for DM. This review provides an overview of the technological advances in research on DM-relevant stem cells and their integration into microphysiological environments as provided by the organ-on-a-chip technology.

Contents

Abbreviations: μCCA, Microscale cell culture analog; μMUX, Microfluidic input/output multiplexer; 3D-μFCC, 3D microfluidic channel-based cell culture system; ADME processes, Absorption distribution metabolism excretion processes; APC, Antigen presenting cell; Arg, Arginine; Asp, Aspartate; BAT, Brown adipose tissue; BMI, Body mass index; CD (e.g. CD4+), Cluster of differentiation (e.g. cluster of differentiation 4+); CITR, Collaborative Islet Transplant Registry; CVB4, Coxsackievirus B 4; CYP, Drug-metabolizing cytochrome P450; DM, Diabetes mellitus; DN, Diabetic nephropathy; DPP-4, Dipeptidyl peptidase; DR, Diabetic retinopathy; dsRNA, Double-stranded RNA; EBI2, Epstein-Barr virus induced gene 2; ECM, Extra cellular matrix; ER, Endoplasmatic reticulum; EV, Enterovirus; FPG, Fasting plasma glucose; GDM, Gestational diabetes mellitus; GIP, Glucose-dependent insulinotropic polypeptide; GLP-1, Glucagon-like peptide 1; GTT, Glucose tolerance test; GWAS, Genome-wide association studies; hASC, Human adult stem cell; HbA1C, Glycated hemoglobin A_{1c}; hESC, Human embryonic stem cell; HIF, Hypoxia inducible factor; hiPSC, Human induced pluripotent stem cell; hiPSC-Hep, Human induced pluripotent stem cell -derived hepatocyte-like cell; HLA, Human leukocyte antigen; HSC, Hematopoietic stem cell; hTERT, Human telomerase reverse transcriptase; IDF, International Diabetes Federation; IFG, Impaired fasting glucose; IFIH1, Helicase c domain-containing protein 1; IFN, Interferon; IFNAR, Type I IFN receptor; IFN-γ, Interferon gamma; IL-1β, Interleukin 1 beta; IL-6, Interleukin-6; IR, Insulin receptor; IR-Mut, Insulin receptor mutation; lncRNA, Long-non-coding RNA; LYP, Lymphocyte-specific tyrosine phosphatase; MCP-1, Monocyte chemoattractant protein-1; MHC, Major histocompatibility complex; MOC, Multi-organ chip; MODY, Maturity Onset Diabetes of the Young; MPCC, Micropatterned co-culture; MPS, Microphysiological system; MSC, Mesenchymal stem cell; MYH, Myosin heavy chain; NOD, Non-obese diabetic; NPC, Non-parenchymal cell; NPSC, Neural progenitor stem cell; OoC, Organ-on-a-chip; PDMS, Polydimethylsiloxane; PDX1, Pancreatic And Duodenal Homeobox 1; PG, Plasma glucose; PHH, Primary human hepatocyte; PPAR-γ, Peroxisome proliferator-activated receptor gamma; PTC, Premature termination codon; PTPN22, Protein tyrosine phosphatase non-receptor type 22; RAFT™, *Real Architecture For 3D Tissue*; REG3A, Regenerating islet-derived protein 3 alpha; SGLT, Sodium glucose co-transporter; SGLT2, Sodium-glucose co-transporter 2; SV40LT, Large T antigen of simian virus 40; T1DM, Type 1 diabetes mellitus; T2DM, Type 2 diabetes mellitus; TCR, T-cell receptor; TEDDY, The environmental determinants of diabetes in the young; TGF-β, Transforming growth factor beta; TLR, Toll-like receptor; TNF, Tumor necrosis factor; TNF-α, Tumor necrosis factor alpha; Tr-cells, T regulatory cells; Trp, Tryptophan; UPR, Unfolded protein response; VLSLL, Very large-scale liver-lobule; VNTR, Variable number of tandem repeat; VP1, EV capsid protein 1; WAT, White adipose tissue.

* Corresponding author at: Department of Women's Health, Research Institute for Women's Health, Eberhard Karls University, Silcherstrasse 7/1, 72076, Tübingen, Germany.
E-mail address: katja.schenke-layland@med.uni-tuebingen.de (K. Schenke-Layland).
[1] Authors contributed equally.
[2] Authors contributed equally.

https://doi.org/10.1016/j.addr.2018.10.010

1. Introduction

1.1. What is diabetes mellitus (DM)?

Diabetes mellitus (DM) is a group of chronic disorders characterized by a dysregulated glucose metabolism. In 2015, one in eleven adults, equaling 415 million people worldwide, suffered from DM making it one of the world's greatest health concerns [1]. The global cost of DM was estimated at approximately 700 billion USD in 2015 alone [1]. In many countries, 5–20% of health care expenditures are spent on diabetes. According to the International Diabetes Federation's (IDF) *Diabetes Atlas 2015*, one in ten adults will be affected by 2040 boosting global health spending to treat diabetes to around 800 billion USD [1].

The physiological regulation of glucose metabolism in the human body is a feedback loop based on endocrine signaling between pancreas, liver, and glucose-consuming tissues. The physiological homeostatic value of blood glucose is about 90 mg/100 ml [2]. Rising levels of blood glucose, as after food intake, stimulate β-cells in the pancreatic islets (also called islets of Langerhans) to produce and release the hormone insulin while simultaneously inhibiting the production of the insulin-antagonist glucagon. Insulin, which is secreted into the blood stream, mediates the uptake of glucose, fatty acids and amino acids in insulin-sensitive tissues, like skeletal muscle, for instance, or the liver, which stores glucose in form of glycogen. As a consequence of declining blood glucose levels, the trigger inducing insulin secretion and glucagon restriction ceases. The further reduction of blood glucose levels results in the activation of glucagon secretion from α-cells. In the liver, glucagon prompts the breakdown of glycogen to glucose, which is released to the blood elevating blood glucose levels until the homeostatic value is reached, which presents a set point halting glucagon production. The reduction of β-cell insulin production or defective responses to insulin in tissues are common characteristics of DM disorders and result in high blood glucose levels, termed hyperglycemia.

Different types of DM disorders are characterized by the underlying mechanisms causing hyperglycemia and can be categorized into three main types: type 1 DM (T1DM), type 2 DM (T2DM) and gestational DM (GDM) [3]. Less common types of diabetes are monogenic diabetes (elicited by a single gene abnormality as compared to the polygenic inheritance of T1DM and T2DM) [4,5] and secondary diabetes (drug- or chemical-induced or arising as a co-morbidity of other diseases) [1,6]. GDM is a condition of progressively increasing insulin resistance during pregnancy [7,8]. Despite the lack of scientific consensus regarding the exact definition of GDM [9], it is agreed that many of the pathophysiological characteristics of GDM correspond to those of T2DM [8]. This review will hence concentrate on T1DM and T2DM with a particular focus on the new opportunities arising from emerging technologies in the fields of stem cell research and microphysiological in vitro models.

1.2. T1DM & T2DM: triggers, courses of disease, similarities & differences between both types of DM

1.2.1. Type 1 diabetes mellitus (T1DM)

T1DM is characterized by the autoimmune destruction of pancreatic β-cells [10]. This complex and heterogeneous disorder is mostly diagnosed in children and adolescents, who usually present a classic trio of symptoms – polydipsia, polyphagia, polyuria – together with hyperglycemia [10]. Incidence and prevalence of T1DM considerably vary among countries [11,12]. The cause of these variations are still unknown and are commonly referred as the "environmental factors" contributing to the disease development [10]. Based on a large number of studies and a comprehensive scientific effort, the current general agreement within the community is that the autoimmune response towards β-cells is triggered by a set of different environmental factors in genetically predisposed individuals [13]. This process is mediated via the activation of autoreactive β-cell-specific helper CD4+ and cytotoxic CD8+ T-cells which infiltrate the islets and destroy the beta-cell mass [13].

One initiating event in T1 diabetes is an impaired negative selection that might occur due to a reduced expression of self-antigen(s) in the thymus and other lymphoid organs during establishment of the central tolerance (Fig. 1A) [14–17]. The negative selection is one of the checkpoints occurring in the thymus during differentiation of thymocytes into mature T-cells; by this mechanism only cells which are non-reactive to self are selected [18]. Self-reactive effector T-cells (CD4+ and CD8+) which have escaped negative selection are activated in the lymph nodes by antigen presenting cells (APCs) that express islet-specific autoantigens (Fig. 1B). The activation of helper (CD4+) and cytotoxic (CD8+) T-cells occurs through the interaction of their T-cell receptor (TCR) with peptide-major histocompatibility complexes (MHC) class II and class I, respectively [19,20]. In addition to self-reactive effector T-cells, there is evidence that alterations in the frequency and function of T regulatory (Tr)-cells have a role in the development of T1 diabetes [21]. These cells are critical for maintaining self-tolerance, as they have the ability to suppress autoreactive T-cells.

Activated self-reactive CD4+ and CD8+ cytotoxic T cells infiltrate the pancreatic islets via vascularization [22]. Once infiltration is

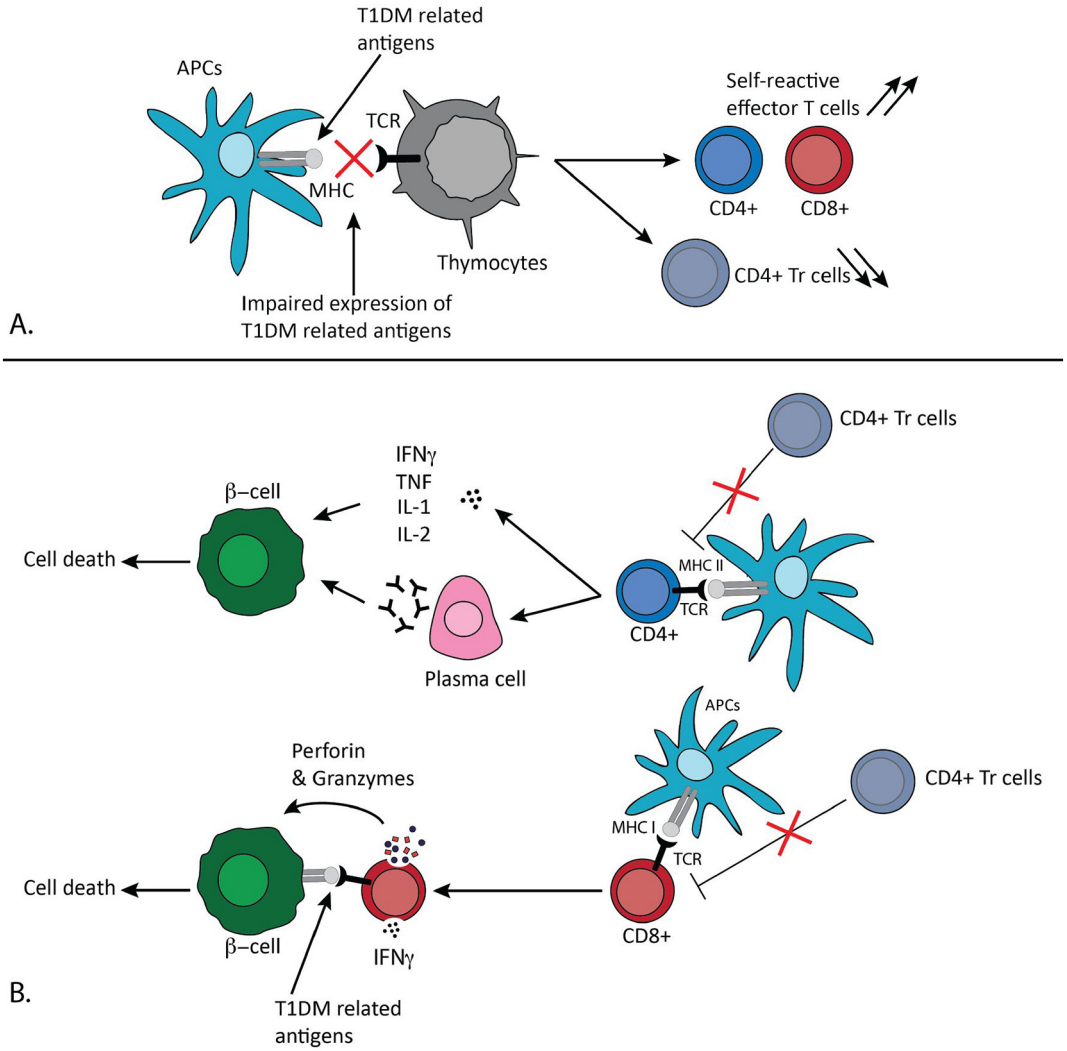

Fig. 1. Simplified mechanism of T1DM autoimmunity. A. Impaired negative selection in the thymus leading to increase in self-reactive effector T-cells (CD4+ and CD8+) and reduced T regulator cells (CD4+ Tr). B. Activation via APCs of T helper cells (CD4+) and cytotoxic T cells (CD8+) through MHC class II and I respectively. CD4+ helper T cells secrete pro-inflammatory cytokines and stimulate auto-antibody production in plasma cells. CD8+ cytotoxic T cells secrete perforin and granzymes. Altogether, these mechanisms induced by T effector cells lead to the destruction of beta β-cells.

initiated, subsequent immune cell entry is facilitated. The islet basement membrane ruptures and highly invasive insultis occurs, leading to β-cells death [23]. Secretion of certain cytokines such as tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), or interleukin 1 beta (IL-1β) by immune cells ultimately lead to β-cell apoptosis [24]. Due to the decrease in β-cell number, the workload on the remaining β-cells is dramatically increased. This can induce apoptosis through a variety of pathways, such as the elevation of stress levels in the endoplasmatic reticulum (ER), where misfolded or unfolded proteins accumulate [25]. Cells react to ER stress by activating the adaptive unfolded protein response (UPR) and if ER stress persists, the UPR can switch from an adaptive response towards a pro-apoptotic response [26] (see [25] for complete review of UPR and T1DM).

1.2.1.1. Genetic predisposition. T1DM is strongly polygenic with over 40 known loci in the human genome, most of them being related to the immune response [27]. This supports the notion of genetics influencing the mechanisms contributing to an aberrant immune response, including an incomplete self-tolerance to β-cell antigens by the adaptive immune system, as well as triggers from a dysfunctional innate immunity [27].

Half of the genetic susceptibility leading to T1DM arises from various mutations within the MHC regions, which includes the human leukocyte antigen (HLA) region on chromosome 6 [28]. The HLAs are a family of homologous proteins that present antigens to T-cells [29]. Class I HLA molecules (HLA-A, HLA-B and HLA-C) present endogenous antigens to CD8+ (cytotoxic) T-cells, while class II HLA molecules (DP, DR and DQ)) present antigens to CD4+ (helper) T-cells. Most T1DM susceptibility occurs in HLA class II DR and DQ loci, particularly in the regions encoding the beta-chain genes (DRB, DQB) [30]. For example, replacing the amino acid at position 55 by any other amino acid than aspartate (Asp) is an important trigger of T1DM pathogenicity [31]. The loss of Asp residues may lead to altered bindings to antigenic epitopes, resulting in an ineffective tolerance induction and autoimmunity [31]. For a complete review on HLA regions associated to T1 diabetes, see review [32].

Different non-HLA regions have been associated to T1 diabetes, however their contributions are weaker compared to HLA regions. As previously mentioned, one potential trigger leading to autoimmunity is the failure to establish self-tolerance. Interestingly, it appears that the variable number of tandem repeats (VNTRs) polymorphism located before the insulin gene leads to an incomplete immune tolerance to insulin [33]. It has been shown that short VNTRs (26 to 63 repeats) predispose to T1DM, whereas long VNTRs (140 to 210 repeat) are protective [34]. Further in vitro studies have demonstrated that VNTR length directly affects the expression of insulin in the thymus and is specific to the native

insulin promoters [35]. Finally, other evidence indicates that the expression of tissue-specific protein in the thymus is crucial for self-tolerance [36]. Altogether, short VNTR variants reduce INS expression in the thymus, impairing a proper tolerance to insulin. The protein tyrosine phosphatase non-receptor type 22 (PTPN22) is an additional immune regulator associated with defective self-tolerance. PTPN22 encodes for the lymphocyte-specific tyrosine phosphatase (LYP) which is a negative regulator of T-cell kinase signaling determinant for host defense and self-tolerance [37]. The mutation of arginine (Arg) at position 620 to tryptophan (Trp) has an inhibitory effect on LYP [37]. As a consequence, downregulation of TCR signaling occurs which in turn interferes with proper tolerance induction in the thymus or the periphery [37].

Deficiency within the innate immune response is also associated to T1 diabetes. The Epstein-Barr virus induced gene 2 (EBI2) genes regulate a network related to innate immunity[38] and helicase c domain-containing protein 1 (IFIH1) loci belong to a family of helicases that can initiate an interferon response upon sensing viral double-stranded RNA (dsRNA) [39]. The association of IFH1 with T1 diabetes supports the hypothesis that virus infections have a triggering role in the disease initiation (see below).

1.2.1.2. Environmental triggers. A study on identical twins in 1972 was one of the first to indicate the importance of the environmental context of T1 diabetes [40]. A concordance of only 30–50% in the overall monozygotic twin population was observed, implying a substantial environmental component to contribute to the development of T1D.

Viral infections have caught attention as potential environmental trigger of T1 diabetes. From an evolutionary point of view, viruses and the immune system are closely connected, as virus infections have stimulated the development of the human immune system and promoted the diversity observed in the MCH complex, T-cell responses and B-cell antibody production [41]. It is important to note that current scientific reports have failed to demonstrate the direct causality between viruses and development of T1 diabetes in humans. Nevertheless, indirect associations have been compiled over the last decade suggesting a link between viruses and T1 diabetes, including the enteroviruses, herpesviruses, rotaviruses, retroviruses and picornaviruses [42]. From all potential candidates, the prime suspects as hypothetical trigger of T1 diabetes are enteroviruses (EVs) [43].

The first studies investigating the link between EV infection and T1 diabetes failed to associate the presence of islets autoantibody and viral RNA and often reported contradictive results [44–46]. In smaller scale cohort studies, Krogvold et al. obtained pancreatic biopsies from diabetes patients undergoing pancreatic resections [47]. The processed tissues did reveal the presence of EV capsid protein 1 (VP1) but less than 2% of the Langerhans islets were positive and the level of RNAs was very low [48]. These results suggest that T1 diabetic patients were subjected to a lower grade viral infection rather than an acute viral infection. Another approach to investigate if EV infection is linked to T1 diabetes was the development of EV infection animal models. Gallagher et al. transplanted immunodeficient mice with human islet grafts and infected them with type CVB4 enterovirus [49]. Upon infection, multiple interferon-stimulated genes were induced and human transplanted β-cells became dysfunctional with a deficiency in insulin production leading to the development of diabetes. In a more recent study, proteomic alterations were characterized in cultured human islets upon in vitro CVB4 EV infection providing new insight of T1 diabetes etiology [50]. Following viral infections, specific protein expression profiles were modulated and interestingly the insulin levels decreased [50]. The most significant downregulation was observed in the REG3A (regenerating islet-derived protein 3 alpha), which is potentially linked to β-cell neogenesis and insulin production [51]. Recently, new evidence has highlighted the involvement in T1 diabetes of the interferon (IFN) pathways, or so-called, "interferon signature" which refers to the expression of genes that are known to be regulated by the IFNs [52]. The hypothesis emerging involves the activation of the host antiviral type I IFN immune signaling pathways upon viral infections [52]. It leads, in part, to the translation and synthesis of IFN-β, which further interact with type I IFN receptors (IFNAR1 and 2), enhancing inflammation [52] and is believed to be the trigger towards autoimmunity [53]. Lastly, it has been shown that IFNAR1 deficiency is protective against diabetes development [54].

The combination of cohort studies, animal models and genomics has provided better insight into the potential mechanism of viral infection leading to T1 diabetes. This understanding enables the adjustment of the experimental platforms for larger cohort studies. For instance, in a recent study by Honkanen et al., long-term sample collection was performed from 129 children who developed signs of beta-cell dysfunction and controls. This prospective study analyzed in a time-depend manner the association between EV infection and initiation of the β-cell damaging process [55]. The data suggested that EV infection is associated with islets' autoimmunity development with a certain time lag of several months, likely by damaging β-cells [55].

In recent years, further environmental triggers or protective factors for T1DM have been suggested: Studies investigating the role of intestinal microbiota, for instance, found that healthy individuals have a different microbiome compared to prediabetes or T1 diabetes patients [56,57]. Whether these differences are a cause or a consequence from T1 diabetes needs to be further determined. For a comprehensive overview on intestinal microbiota and T1 diabetes see review [57]. Other studies propose that life events which induce a high insulin demand are accelerating β-cell damage [58]. These life events could, for example, be rapid growth, overweight, puberty, lack of physical activity, trauma or infection. In the last decade, epigenetic factors emerged as possible contributors, in particular with recent evidence suggesting that DNA methylation plays a role in T1 diabetes [59]. Finally, various dietary factors, such as breastfeeding, cow´s milk, vitamin D or polyunsaturated fatty acids have been associated with T1DM [58,60–62]. However, most of the findings towards a link between diet and T1 diabetes are contradictory and further research is required.

1.2.1.3. Treatment options for T1DM. In 1923, insulin was discovered by Banting and Best [63]. During the next ninety years of research, different insulins and analogs were successfully synthetized and brought to the market [64]. Gradually, insulin has been structurally modified to improve its properties: short or long-acting insulins were generated with increased stability and various absorption profiles. Insulin therapy is currently the principal and mostly used treatment against T1DM. For a successful insulin therapy, control of the carbohydrate intake and anticipating physical activity is required. The choice for the right insulin analog is made depending on the age, physiology and frequency of hypoglycemic events [65]. Besides insulin injection, pramlintide has been approved by the FDA to treat T1DM patients. It is an amylin analog, which delays gastric emptying, decreases glucagon secretion and enhances satiety, reducing the risk of hypoglycemic events [66]. When the control over glycemia cannot be achieved, the use of an adjuvant therapy is possible including metformin, glucagon-like peptide 1 (GLP-1) receptor agonists, dipeptidyl peptidase (DPP-4) inhibitors or sodium-glucose co-transporter 2 (SGLT2) inhibitors [67]. Despite intensive insulin therapy and its combination with other pharmaceuticals, many adult diabetic patients do not manage to fully control their glycemia and suffer from hypoglycemic events. This can be highly dangerous, involving psychological distress and, in serve cases, an increase in mortality [68].

Far-reaching alternatives are pancreas and Langerhans islets transplantations to restore regulation of blood glucose levels [69]. A complete pancreas transplantation is a major surgical procedure with consequential risks of thrombosis as well as consequences from the lifelong immunosuppressive therapy to prevent graft rejection [70]. In addition, pancreatic transplantation is privileged to T1 patients that are also undergoing a renal transplantation, following a renal transplantation, or patients that suffer from severe hypoglycemic events despite an

intensive insulin therapy [65]. Due to the low availability of transplants and the need of heavy immunosuppression, a very small number of pancreatic transplantations are performed, insignificant compared to the size of the diabetic population [70]. Langerhans islets transplantation is considered a safe procedure with much lower associated risks compared to any other solid organ transplantation according to the Collaborative Islet Transplant Registry (CITR) [71]. The islets are infused through the portal vein and then engrafted in the liver (distal portal triad) [69]. In the long-term, 50–70% of patients achieve insulin independence at 5 years post-transplantation – success rates similar to whole pancreas transplantation [72].

In summary, insulin therapy provides control over the glycemia for the majority of patients. However, it comes along with constant restrictions on daily's life quality. Additionally, a non-negligible number of patients are still subject to hypoglycemic events which can potentially leading to severe complications in the long-term. Complete pancreas transplantation and islets transfusion are invasive alternatives that can provide insulin independence. Yet, patients still need lifelong treatment and the relieving effect often only lasts for a few years. To improve therapy for T1DM patients and to develop new approaches, there is hence a strong need for further research towards the understanding and characterization of the factors leading to T1DM and its underlying mechanisms.

1.2.2. Type 2 diabetes mellitus (T2DM)

T2DM is the most prevalent type of DM accounting for 91% of all adult (i.e. 20–79 year-old) diabetic patients [1,73]. The term 'T2DM' is therefore frequently mentioned in conjunction with "global health burden" [73], "epidemic" [74] or "scourge of our time" [74]. Compared to T1DM, T2DM is extremely multifactorial resulting in highly individual pathophysiology. A sub-classification of T2DM is hampered by varying contributions of associated risk factors, symptoms and underlying pathophysiological mechanisms as well as disease progressions. Moreover, this variation complicates the diagnosis of T2DM and it is estimated that around 30% of T2DM-patients remain undiagnosed [73].

1.2.2.1. Pathogenesis. Even though T2DM can have many facets, a progressive insulin resistance, β-cell dysfunction and a state of chronic inflammation constitute the common key culprits of a defective control over blood glucose levels. Usually, the first anomaly indicating a potential development of T2DM is the loss of insulin sensitivity in glucose target-tissues [75]. To complete the clinical picture of T2DM, an additional loss of the pancreas' insulin secretory activity is necessary [74–76]. The importance of each of these two abnormalities for the development of T2DM was discussed for a long time: past considerations focused on the insulin resistance being the main defect resulting in secondary β-cell exhaustion due to an urge to compensate the increasing insulin need [74,77]. Nowadays, it is well established that both abnormalities can indeed contribute equally to T2DM pathogenesis [65]. For some patients, it is even a progressive loss of secretory pancreatic activity, which is the initial contributor to T2DM [64].

Even though progressive insulin resistance and impaired insulin secretion are the pivotal abnormalities of T2DM [64], there are at least six more defects that, either individually or in any combination with the other abnormalities, play a part in contributing to the prevailing chronic hyperglycemia [73,75,78]. Taken together, these eight dysfunctions (Fig. 2) were dubbed "the ominous octet" by Ralph A. DeFronzo in 2009 [75].

The mechanisms behind impaired insulin signaling and insulin resistance are complex. In brief, they can mostly be ascribed to an ectopic lipid metabolite accumulation in skeletal muscles and the liver. This lipid accumulation is usually resulting from imbalances in nutrient storage pathways due to a chronic excess of dietary energy. As insulin resistance occurs in skeletal muscle tissue first, all of the ingested glucose is unloaded on the liver which is therefore increasing the hepatic de novo lipogenesis and hyperlipidemia. As a consequence, macrophages are recruited to white adipose tissue (WAT) to increase lipolysis processes, which in turn further enhance the hepatic lipogenesis [79,80]. Moreover, WAT-lipolysis stimulates fasting as well as postprandial hepatic gluconeogenesis resulting in glucose overproduction despite hyperinsulinemia [81,82]. However, insulin resistance is not limited to the two main glucose metabolizing tissues, muscle and liver, but it also occurs in other tissues, as adipose [83,84], vascular [85], brain [86] and intestinal tissues [87], for instance.

On part of pancreas-associated pathophysiology, changes in β- and α-cell functionality add to the chronic hyperglycemia. The roots for β-cell failure can be as multifarious and individual as the clinical picture of T2DM; among them are advanced age [88,89], β-cell exhaustion resulting from insulin resistance compensation [75,90,91], genetic anomalies causing β-cell fragility [92,93], and stimulation of inflammatory pathways [94,95]. Failure of β-cells is usually caused by two different defects: on the one hand, T2DM patients seem to lose around 30–40% [96] of their β-cells by apoptosis [97,98] and dysregulated autophagy [99,100]; on the other hand, the functionality of the remaining β-cells progressively becomes impaired. Two different mechanisms may be responsible for the β-cells' functional constraints: firstly, debilitated glucose sensing might cause the diminished secretory function of the remaining β-cells [101]. Secondly, a loss of their insulin secretion activity might be attributable to a de-differentiation of the mature β-cells to a progenitor-like state as it was found in rodent studies [102,103]. Pancreatic α-cells contribute to the hyperglycemia by increasing their glucagon production along with the increased hepatic glucose production described above [75,104,105].

Further members of the T2DM "ominous octet" adding to chronic hyperglycemia are the brain, the kidney and the small intestine. With abnormally increased serotonin and lowered dopamine levels, the brain pleads blameworthy of impaired satiety-signaling which plays into the hands of weight gain, obesity and insulin resistance, and thus implicitly aggravates hyperglycemia [73]. In the kidney, the threshold for glucose excretion is increased by an upregulation of the sodium glucose co-transporters (SGLTs) 1 and 2. As a consequence, renal proximal tubular glucose reabsorption is increased [106]. Finally, the secretion of the incretin hormones GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) by the small intestine can be gradually reduced in T2DM patients; this defect severely affects insulin secretion because the physiological action of these two hormones, summarized as "incretin effect", should actually augment the postprandial insulin production. This amplification cascade cannot be passed through when GLP-1 and GIP are absent [107,108]. However, more recent findings suggest that the ominous octet should be extended to the "decadent decuplet" including vascular defects as well as systemic inflammation [73]. In arterial vasculature, insulin resistance disrupts the phosphatidylinositol 3-kinase pathway that usually causes vasodilation. By diminishing the production of nitric oxide by endothelial cells, the lack of insulin induces vasoconstriction. This effect leads to decreased capillary recruitment and decreased total blood flow [85]. Moreover, systemic inflammation is involved in insulin resistance and hyperglycemia: as mentioned before macrophage infiltration into WAT stimulates increased lipolysis in response to hyperlipidemia. Moreover, the activation of pro-inflammatory cytokines such as tumor necrosis factor (TNF) and interleukin-6 (IL-6) represents a key player in insulin resistance. Elevated levels of IL-6, for instance, trigger increased gluconeogenesis by the liver and particularly contribute to hepatic insulin insensitivity [73].

1.2.2.2. Risk factors. That the global boost of T2DM roots in the increasing prevalence of obesity is safe to assume [74,110]; obesity (i.e. a body mass index (BMI) ≥ 25 kg/m^2) is the most prominent risk factor preceding T2DM, resulting in the combined denomination "diabesity" [110]. The roots of overweight and obesity can also entail T2DM; among them are lifestyle factors most prominently the burden of chronic excess dietary energy with unhealthy diet and physical inactivity [73].

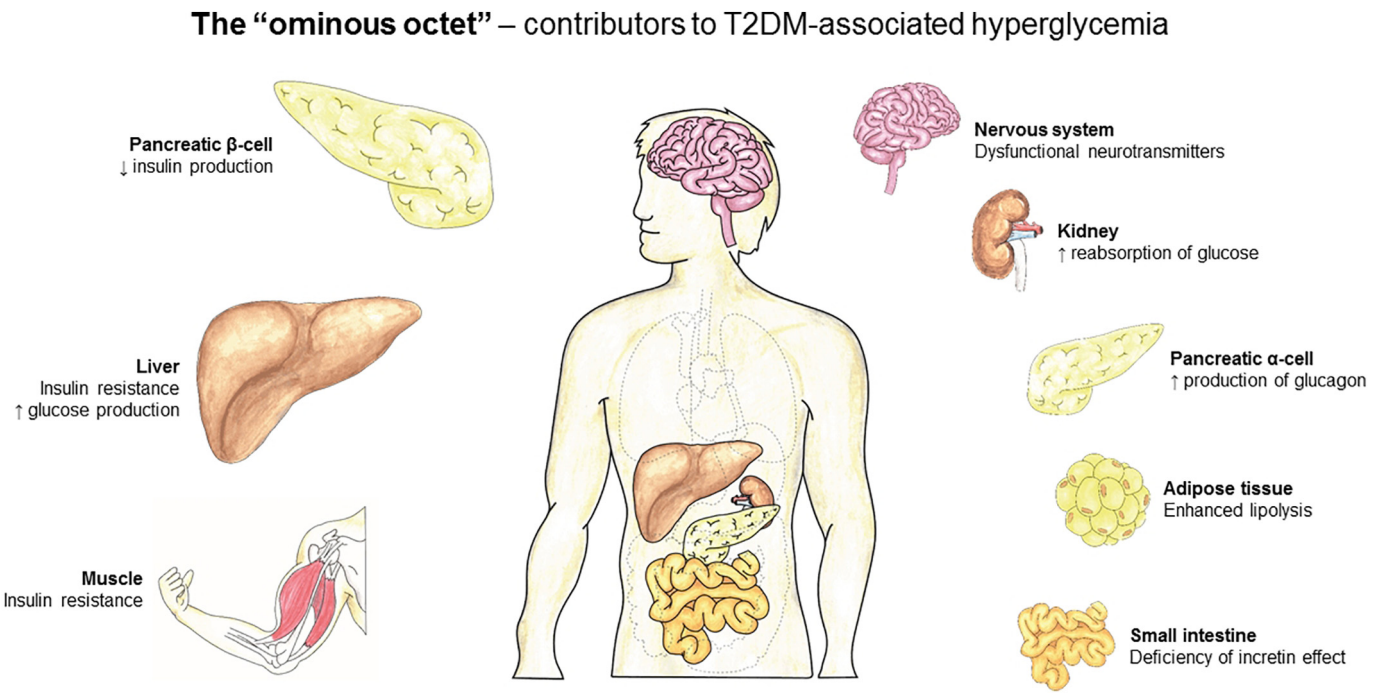

Fig. 2. Overview of the multifactorial character of type 2 diabetes mellitus (T2DM). There are eight different defects, also termed "the ominous octet" [73,75], contributing both individually or in any combination to T2DM-asscociated hyperglycemia. The two pivotal abnormalities leading to increased blood glucose levels are (i) decreased insulin secretion by pancreatic β-cells, and (ii) the development of insulin resistance in the liver, skeletal muscle, and other peripheral tissues. As a consequence, hepatic glucose production is increased (iii). Additional effects which cause aggravation of T2DM (and might as well be evoked by one of the other T2DM-deficiencies) include (iv) failure of neuronal transmission of insulin-associated appetite-suppressing signals leading to weight gain, (v) increased renal reabsorption of glucose which maintains hyperglycemia, and (vi) increased secretion of glucagon by pancreatic α-cells. Moreover, (vii) increase of insulin resistance in adipocytes results in enhanced lipolysis, and therefore enhanced levels of free fatty acids. This, in turn, deteriorates adipose insulin resistance. An (viii) incretin deficiency in the small intestine leads to further failure of β-cells.

Even independent of body weight, some diets are associated with a higher risk for diabetes than others: as shown by clinical cohort studies in the US, a diet mainly rich in refined grains, red or processed meat, sugar and sugar-sweetened beverages predisposes more to T2DM development than diet based on whole grains, veggies and fruit, white meat and coffee [100,101]. Further impacts of lifestyle on T2DM risk include sleep and sleep duration: too little (≤5 h/day) or too much sleep (≥ 9 h/day) [111,112] and an irregular daily routine as in rotating night shift work [113] were also associated to T2DM prevalence.

These lifestyle factors not only put people at risk to develop T2DM, but also the development of a condition called prediabetes, which is not uncommon. As indicated by its name, prediabetes is often a precursor of T2DM, especially its facet of impaired fasting glucose (IFG). It is characterized by blood glucose levels higher than usual during fasting periods, but the threshold defined for diabetes (110–126 mg/dL) is not exceeded [98]. An early detection of prediabetes combined with a lifestyle intervention can very likely prevent the progression to T2DM [114].

Besides lifestyle and nutrition, genetic predisposition plays an important role in T2DM development. Even though it is known that T2DM is heritable, it is a tough challenge to identify all genes associated with T2DM because of its complex polygenic character [73]. Moreover, sex and gender differences may also affect T2DM predisposition, pathogenesis and accompanying complications: despite obesity being more typical for women, T2DM is slightly more often diagnosed in men than in women [73,115]. Besides the different gender-specific impacts of (epi-) genetics, endocrine signaling and sex hormones acting on metabolism and homeostasis might also be involved in T2DM development and presentation [115]. Adding to these biological factors, T2DM is also supposed be influenced by psychosocial components [115].

1.2.2.3. Clinical diagnosis of T2DM. The most prominent and equally effective screening tools for a T2DM diagnosis are measurements of plasma glucose (PG) levels and glycated hemoglobin A_{1c} (HbA1C). PG levels can be measured in different states: most common are measurements in a fasting state, i.e. at least 8 h after the last caloric intake [fasting PG (FPG); diagnosis of T2DM: FPG≥126 mg/dL], or 2 h after the consumption of 75 g anhydrous glucose dissolved in water [two-hour PG; diagnosis of T2DM: two-hour PG≥200 mg/dL] [78,116]. HbA1C tests assess a patient's average blood glucose levels over a period of 2–3 months by chronically marking hyperglycemia [117].

1.2.2.4. Treatment options for T2DM. To date, there is no cure for T2DM, but there are treatment options to control the severity of the disease. Due to the multifactorial character of T2DM, the optimal disease management is a combination of a variety of interventions including close interaction between patients and physicians, a lifestyle change, pharmacological therapy as well as a close and regular diabetes control [78,118].

A lifestyle intervention is not only the best prevention strategy for T2DM [110], but a more active lifestyle and healthy diet significantly diminished the risk to develop T2DM in prediabetes patients [114]. Even diabetic patients are advised to lose around 7% of their body weight because of a positive outcome on blood glucose levels, blood pressure and cholesterol values [78,114]. Another key treatment is a pharmacological therapy of T2DM and hyperglycemia. Due to the heterogeneity of T2DM, medication options are tremendously miscellaneous and several medicines have to be combined to optimally treat each patient's individual form of T2DM [75]. For T2DM, the most prominent anti-diabetic drug is metformin, which is an oral drug and usually the first choice to curb blood glucose levels in T2DM patients [78]. Metformin belongs to the biguanides, a group of anti-diabetic agents found to counteract T2DM-induced hyperglycemia by decreasing hepatic glucose production [119]. Compensating the lowered incretin effect caused by a decreased intestinal secretion of GLP-1 and GIP, incretin mimetics are an important approach to treat T2DM. Substances as GLP-1 receptor agonists,

for instance, were shown to help regaining enhanced glycemic control in T2DM-patients [120]. To block the increased renal tubular glucose reabsorption, drugs based on inhibiting mechanisms for SGLT2 hold promise; among them are dapagliflozin, canagliflozin, and empagliflozin [121]. Similarly, dipeptidyl peptidase (DPP)-4 inhibitors are emerging as a way to improve the glycemic control by modulation of the incretin effect [122].

Even a combined basal insulin administration can be required: Especially for patients that did not respond to other anti-hyperglycemic drugs, the supplementary intake of insulin analogues is part of the treatment strategy [123,124]. The list of pharmacological treatment options is long and this paragraph highlighted only a few of many possible medications. Chaudhury and Mirza put together a detailed clinical review on antidiabetic drugs for T2DM treatment [78].

1.2.3. Comparison

Even though the naming indicates a close relation between T1DM and T2DM, the two previous sections with a focus on the two DM types individually, showed that the two disorders seem actually more distinct from than similar to each other – except for their main symptom, hyperglycemia.

In T1DM, hyperglycemia is evoked by autoimmune destruction of β-cells while in T2DM metabolic dysfunction is the cause of elevated blood glucose levels (Fig. 3). The main causes underlying DM are also T1- and T2-specific. Despite genetic predisposition playing a significant role in both types, it plays a more important role in T1DM development: Genetic factors, potentially in combination with triggers like certain viral infections or dietary influences, lead to the autoimmune destruction of pancreatic β-cells and, consequentially, to a lack of insulin and elevated blood glucose levels. The main risk factors for developing T2DM are obesity, and lifestyles characterized by physical inactivity and unhealthy diets in general, as well advanced age and, to a certain degree, genetic predisposition. However, as explained in detail before, T2DM with its underlying mechanisms is extremely heterogeneous making it difficult to determine one fixed definition of the course of disease; the mechanisms leading to hyperglycemia are as individual as each patient. Hence, Fig. 3 displays only one mechanism of many underlying T2DM: in this specific case, a progressive insulin resistance precedes β-cell failure provoking elevated blood glucose levels. Another difference between T1DM and T2DM lies in the onset of the disease: while the onset of T1DM in most cases occurs before the onset of puberty, T2DM is rather associated with advanced age. Albeit, as distinct the contributors to chronic hyperglycemia are in T1DM and T2DM, the co-morbidities arising through the sustained excess of glucose on the blood are considerably alike.

1.3. Chronic and acute complications arising through diabetes

T1DM and T2DM are associated with a wide range of complications, mainly occurring due to chronic elevation of the blood glucose level. Most severe co-morbidities thereby are caused by damages to the micro- and macrovasculature [125]. Microvascular complications include diabetic retinopathy, nephropathy and neuropathy; while macrovascular complications manifest through myocardial infarcts, strokes and heart failures. Some of the major complications of T1DM are retinopathy (47% risk), nephropathy (17% risk), and cardiovascular diseases (14% risk) [126]. In the case of T2DM, a study from 2013 reported 53.5% of all analyzed T2DM patients (66,726 in total) to suffer from microvascular comorbidities, and 27.2% to be affected by macrovascular complications [127]. The major contributors to diabetic complications are (i) hemodynamic changes such as blood pressure or salt/fluid balances, (ii) metabolic changes including glycemic imbalances and lipid metabolism switch, as well as (iii) genetic susceptibility [128]. These factors are causing drastic changes on a cellular level in gene modulation, energetic profiles and protein expression, leading to

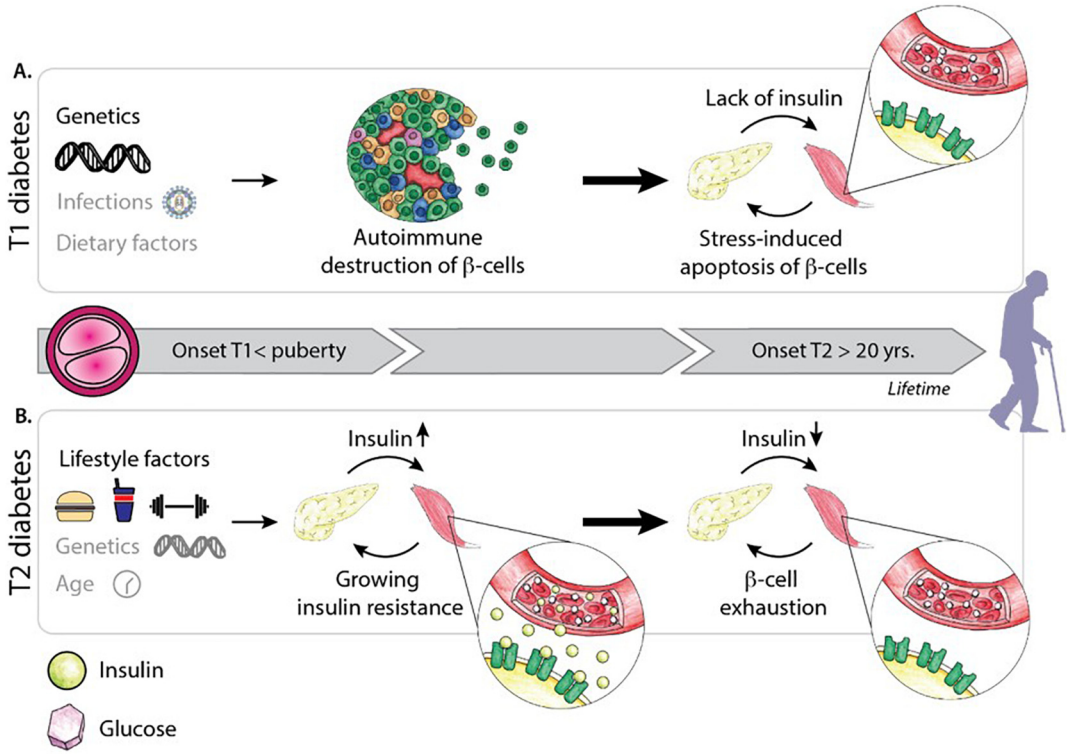

Fig. 3. Comparison between T1DM and T2DM. A. T1DM: The main contributors to disease development (predominantly genetic factors) are leading to the autoimmune destruction of β-cells. Due to the lack of insulin production, the workload of the remaining β-cells is increased, long-term leading to stress-induced apoptosis. B. T2DM: The most important risk factor of T2DM is obesity which is usually the result of an unhealthy lifestyle. Moreover, genetic predisposition and the human's age play a role. The presumably most common type of T2DM is characterized by a developing insulin resistance of glucose-metabolizing tissues as skeletal muscle or liver, for instance. Despite the pancreas' increased production of insulin, the hormone is not able to enter the cells. Simultaneously, or as a result of exhaustion, β-cells are destroyed and diminish the insulin production.

further cellular dysfunction and recruitment of the immune system [128].

Here we describe briefly the key elements leading to diabetic complications; for a complete review, please refers to Forbes et al. [125]. Vascular endothelial cells are particularly sensitive to hyperglycemia as glucose can enter uncontrolled by insulin presence through diffusion [129]. Intracellular accumulation of glucose alters different pathways leading to a change in the endothelial redox state. As results, an increase in vascular permeability and contractibility occurs, which in turn stimulates extracellular matrix (ECM) synthesis, thickening of the basement membrane and activation of various cytokines and adhesion molecules. Presentation of these adhesion molecules leads to further interactions with immune cells and blood platelets, promoting a pro-inflammatory and pro-coagulant state. Altogether, vascular injury, oxidative stress, inflammation and alteration in the hemodynamic balances due to chronic hyperglycemia play an important role in the development of diabetic vascular complications [130].

For instance, hemodynamic changes are an important initiating factor of nephropathy, which is the most common complication in T1DM patients. Hemodynamic changes induce an increase in glomerular filtration rate. The triggered hyperfiltration exposes the kidney-resident cells to an abnormal level of glucose, fatty acids, proteins, amino acids, growth factors and cytokines, which triggers a number of pathological pathways eventually causing renal failure.

Changes in blood vessel integrity are moreover responsible for the development of retinopathy: Hyperglycemia leads to intramural pericyte death and thickening of the basement membrane, which in turn alters the blood-retinal barrier and its vascular permeability. Resulting hypoxia leads to ischemia and subsequent degeneration or occlusions in retinal capillaries which are responsible for vision impairment.

Neuropathy is not only associated with vascular but also with non-vascular abnormalities [131]. In addition to pericyte loss and basement membrane thickening, decrease in capillary blood flow leads to an attenuated perfusion of the never fibers and subsequent hypoxia. As a consequence, axonal thickness increases and, in some cases, neurons can decease. Neuropathological clinical manifestations are very diverse and encompass both the somatic and autonomic divisions of the peripheral nervous system; for example, motor system dysfunctions accompanied by a loss of sensory perception and pain, orthostatic hypotension which is the inability to adjust heart rate and vascular tone to maintain blood flow in the brain, or gastroparesis due to an impaired innervation of the gastrointestinal system.

Macrovascular complications, such as cardiovascular disease, are usually initiated via the premature deposition of atherosclerotic plaques. As described above, dysfunctional endothelial cells allow subsequent binding of immune cells, including macrophages and T-cells leading to cell proliferation and matrix deposition. Over time and through complex pathways, atherosclerotic plaques are generated within the blood vessels, which destabilize proper blood flow and can rupture, causing myocardial infarction or strokes. A variety of cell populations show metabolic abnormalities including coronary endothelial, fibroblasts, cardiomyocytes and smooth muscle cells [132]. Smooth muscle cells (SMCs) are of interest as they are critical effectors of vascular function: they switch between a contractile and relaxed state to control vascular flow [132]. A pathological deregulation of vascular flow can lead to tissue ischemia and ultimately cell death. Diabetic cardiomyopathy is a myocardial dysfunction that can be observed in diabetic patients in the absence of hypertension or other classical cardiovascular risk factors [133]. Hyperinsulinemia, hyperglycemia and insulin resistance are the main causes of cardiac insulin resistance and induce various metabolic dysfunctions, which in turn result in cardiac stiffness, hypertrophy and fibrosis, described as the first stage of diabetic cardiomyopathy [134]. The second and final stage of the disease is characterized by significant cardiac remodeling resulting in a marked deterioration of the diastolic function.

2. In vivo research on diabetes mellitus

2.1. Human cohort studies

Research on diabetic patients would be the most straightforward way to understand the underlying mechanisms of DM and develop new therapeutic approaches. Obviously, the possibilities for human in vivo research are highly limited due to a number of ethical concerns [135]. Despite the complexity and restrictions imposed by the use of human models, cohort studies have been responsible for some of the major breakthroughs in T1DM and T2DM genetics [136,137]. In the 70s, the first linkage studies revealed the key involvement of the MCH in the susceptibility of T1DM [138]. Technological advances in genetic sequencing have allowed the mapping of candidate genes, uncovering some of the complexities of the immune dysregulation in T1 diabetes. Genome-wide association studies (GWAS) that started in 2007 have largely facilitated the identification of genetic factors in large cohorts with complex-trait genetics, such as in T1DM and T2DM [139]; outcomes from the five major GWAS in T1 diabetes and T2DM GWAS from the last decade have been summarized by Polychronakos et. al [27] and Visscher et al. [139], respectively. The impact for the newly identified loci in T1DM was disappointing, with weaker influence on disease compared to the established loci from HLA genes, INS or PTN22. Moreover, only a few of the new loci have been fine-mapped to a specific variant or specific gene. In contrast, the T2DM GWAS delivered significant advances in understanding the involved genes [139]. GWAS have identified more than 100 loci associated with T2 diabetes and over a third have been connected to specific genes, through which the effects on diabetes risks are mediated [139].

Human cohort studies have also been employed to identify the contribution of different environmental factors involved in T1DM disease progression on genetically predisposed children [140–143]. In 2004, the comprehensive TEDDY (The environmental determinants of diabetes in the young) study aimed to collect information on a wide variety of environmental factors on more than 7000 children [143]. It led, in part, to a range of publications reporting the associations between presence of auto-antibodies with T1 diabetes progression [143]; child growth with auto-antibodies apparition during early childhood [144]; ages, sex and auto-antibodies [141]; or first infant formula and disease progression [140]. By pooling the data of seven published cohort studies, a recent meta-analysis succesfully revealed a link between diabetes and glaucoma [145]. Similarly, meta-analyses of T2DM human cohort studies reported a significant correlation between an increased risk of atrial fibrillation and T2 diabetes [146], protective effects of fruit and vegetables [147] as well as coffee, whole grains and nut consumption [148], the effect of quality and quantity of sleep on T2DM risk [112], and the importance of physical activity in prevention of prediabetes progression [114]. Although human cohort studies have provided evidence of the genetic and environmental contributions in both T1DM and T2DM, these studies are reduced to investigating if a specific association is statistically significant without elucidating the associated pathophysiological processes.

2.2. Animal models for diabetes research

Due to the unacceptable risks of experimental in vivo interventions in humans, the majority of diabetes research is performed using animal models. In the last decades, a variety of diabetic animal models have been developed for specific applications. The advantages and limitations of those models have been comprehensively discussed in a recent review by King et al. [149].

A typical example is the non-obese diabetic (NOD) mouse model; the most widely used animal model in T1DM. It has been subjected to numerous successful experimental interventions, a majority of which have later failed in human clinical trials; once more highlighting the important differences in pathogenesis between mice and human [150].

Due to the direct linkage to obesity, T2DM research often reverts to obese animal models, such as monogenic, polygenic or diet-induced models, depending on the respective scientific question. Especially for pre-clinical DM research focusing solely on the lack of compensation to insulin resistance, animals with a diminished β-cell functionality evoked by dysfunctional β-cells or reduced cell mass have been utilized [149].

Although a variety of animal (mostly rodent) models for T1DM and T2DM research have been developed and widely employed in mechanistic studies, the translation to humans has been mostly unsuccesfull. Due to the complexitiy and the multifactorial character of DM as well as species-specific mechanisms, the identification and prediction of pathological pathways in human is often simply not possible based on animal data. Animal and human pathophysiology feature significant differences, such as a species-specific glucose regulation: described by Charukeshi et al. [152]. Moreover, the majority of animal models are developed to address one specific aspect of DM and do not represent the multiple other factors involved [149]. Due to the inabilitiy to recreate human DM in animal models, there is a strong need for new and advanced in vitro models that enable the recapitulation of the complex physiology of the human body [153]. Based on advances in and the combination of stem cell technology, tissue engineering, and microfabrication, promising new approaches and systems have been introduced in recent years.

3. In vitro research on diabetes mellitus

3.1. Requirements for the establishment of in vitro models

3.1.1. Cell sources: from animal primary cells to SCs

Establishing in vitro models requires an adequate choice of cell type and source, which enable the systems to model molecular mechanisms representative to human in vivo physiology. Patient-specific cells are a great tool to study disease etiology at the molecular levels, which eventually can lead to the development to new therapies. Primary cells isolated from biopsies, for instance, have been widely used in cancer research, whereby patient-derived tumors are cultured for drug screening purposes [154]. However, primary human cells or tissues are typically only available in small quantities and are therefore unsuitable for high-throughput experimental set ups. A solution for the limited availability is to derive immortalized cell lines from primary cells using specific genetic manipulations. However, such cells often have the drawback of losing their specific phenotypes if continuously grown in culture.

Human stem cells have been of growing interest for in vitro models, disease modelling, and cell-based therapy, including human adult stem cells (hASCs), human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs). Adult stem cells, such as neural progenitor stem cells (NPSCs), mesenchymal stem cells (MSCs) or hematopoietic stem cells (HSCs) are multipotent cells found in the adult body, where they are part of the intrinsic regenerative process of specific tissues [155]. In contrast to hASCs, hESCs and hiPSCs have the ability to differentiate into all cell types in the body [156]. hESCs are thereby isolated from the inner part of blastocysts and hiPSC patient-cells re-programed to their pluripotent state. The main advantages of hiPSCs are their patient-specificity and their somatic origin, eliminating the ethical controversy often connected to the use of hESCs [157]. Despite their differences in origin, it is usually possible to generate in vitro models from both hESCs and hiPSCs. Nonetheless, differences in the epigenetic memory retention between reprogrammed cells and hESCs are currently debated in the field [158]. Genome-wide profiling studies have pinpointed over a thousand differences in DNA methylations between hESCs and hiPSCs, which could be either due to epigenetic mutations or memory retention occurring during reprogramming [158–160]. Although not all epigenetic variants lead to functional implications, it could be valuable to compare genetic profile and functionality of hESC and hiPSCs derived cells.

One limitation of the use of stem cells can be the stochasticity of the differentiation processes for certain cell type leading to highly heterogeneous populations of cells with various degree of efficiency [161]. In addition, the gold standard in assessing the developmental stage of differentiation is by analyzing transcription factors or surface markers; and only rarely by assessing the functionality of the differentiated cells [162]. Defined protocols and procedures need to be established in order to standardize the differentiation processes, including appropriate cell functionality tests of the differentiated cells.

Studying DM and its molecular drivers on human explants is difficult due to a limited access to relevant tissue samples. The stem cell technology, especially hiPSCs, enable research on patient-specific mechanisms on a molecular biological and cytological level [163–167]. Further, the investigation of stem cells, i.e. undifferentiated cells, provides insights into the contributions of defects which are independent of particular differentiation processes.

One such example is the investigation of underlying mechanisms of insulin resistance, a critical aspect of T2DM, by exploring iPSCs from patients with rare genetic mutations in the insulin receptor (IR). An extremely severe form of an insulin resistance caused by insulin receptor mutations (IR-Mut) leading to a complete (or nearly complete) absence of insulin receptors is Donohue syndrome [165]; thus, a derivation of IR-Mut iPSCs from patients with this condition represents a powerful model to elucidate mechanisms behind genetic determination of insulin resistance and how it affects functional and metabolic characteristics of stem cells. Accordingly, in 2014 Iovino et al. generated iPSCs from patients with Donohue syndrome for the first time; comparing IR-Mut iPSCs to healthy control iPSCs as well as to the respective original fibroblasts indicated genetic insulin resistance not only associates to the differentiation state of the cells but also to their cellular context [167]. Further, the correlation between insulin resistance and mitochondrial dysfunction was characterized. Insulin resistant iPSCs were found to have a higher number of mitochondria, which were, however, smaller in size. Moreover, they showed reduced oxidative activity along with reduced energy production [166]. Thus, insulin resistance and deficient insulin receptor signaling are not restricted to differentiated cells but also alter proliferation and function of stem cells.

The previously discussed iPS-derived models are by far not the only progress of the stem cell technology concerning DM research; yet, compared to these studies on differentiation-independent insulin resistance, all other stem cell investigations can be assigned to a certain tissue and will therefore be discussed in the following in the respective organ-sections.

3.1.2. Technology: from simple culture dish to complex architectures

Apart from an appropriate choice of the cell source, in vitro modeling requires culture technologies that provide a physiologically accurate microenvironment for the cells. Even though conventional 2D monolayer culture has provided valuable contributions to biomedical research for almost a century [168–170], in many cases the obtained results failed to predict in vivo responses, which is especially important looking at drug activities [171,172]. The major limitations of 2D monolayer culture, thereby, is that cells are isolated from their native microenvironment. The creation of more complex 2D-models such as coculture systems and cell patterning provided a first step towards more physiologically relevant models [168]. Even higher complexities and physiological relevance has been achieved by tissue engineering 3D models, which promoted higher levels of cell differentiation and tissue organization. In many cases, this was realized by encapsulation of cells inside hydrogels [173,174], whereby a broad range of natural and synthetic hydrogels were employed with specifically adapted mechanical properties and biofunctionalities [174]. Another very prominent approach of 3D modeling is using organoid technology. Organoids consist of 3D clusters of cells derived from primary or stem cell sources. They

exhibit self-renewal as well as self-organizational capabilities providing them with an outstanding potential to model human organ development as well as pathologies [175,176]. Despite reflecting parts of the complex 3D organization of organs, organoids and hydrogel based 3D tissue models are limited in their capacity to recapitulate the entire in vivo situation. Most importantly, they lack vasculature structures and thereby still rely on traditional static dish culture. Vascularization is necessary to enable a physiological continuous transport of soluble factors (e.g. nutrients, oxygen, cytokines, hormones or drug compounds) to the tissue as well as metabolic (waste) products away from the tissue. Additionally, a controlled perfusion would allow the monitoring and quantification of secreted factors as well as consumption and absorption with a high temporal resolution. Moreover, conventional 3D models most often still lack important tissue-tissue interfaces (e.g. with epithelial layers) and cannot be subjected to controlled mechanical stimuli including shear stress, tension and compression forces.

Overcoming those limitations, the organ-on-a-chip (OoC) technology provides engineered microfluidic platforms which integrate human tissues in a microphysiological environment, thereby combining the advantages of cell culture (human genetic background) and animal models (complex physiology). Hence, those microphysiological platforms emulate crucial parts of in vivo physiology including tissue-tissue interfacing, mechanical cues, spatiotemporal chemical gradients as well as vascular perfusion. Further, the technology empowers a broad spectrum of analysis and imaging tools ranging from real-time monitoring of integrated tissues to metabolic quantification and post-experimental tissue retrieval and examination. Those features give a particular importance to the OoC technology in basic as well as applied research, especially in terms of drug development, toxicological screening, personalized medicine and disease modeling. In fact, one branch of the OoC technology is focusing on establishing multi-organ chips (MOCs) and, therefore, empowers modeling of systemic disease progression, as in DM, for instance, and a more dynamic assessment of absorption, distribution, metabolism, excretion (ADME) processes of a drug candidate. The concepts of multi-organ integration are categorized into either static, semistatic or flexible approaches; especially the flexible modular approach is of high interest since it enables a flexible interconnection of already existing, functioning single-organ modules possibly originating from different laboratories. Yet, a successful interconnection faces several challenges of conceptual (i.e. standardization and scaling), technical (i.e. tight seals and robust connectors) as well as biochemical nature (i.e. appropriate culture media composition) [177].

3.2. In vitro models

In this chapter, in vitro models with the potential to help understand and identify treatment options for DM will be discussed. In particular, a special attention will be turned to the promising capability of the human stem cell technology combined with OoC platforms. However, there is also a significant number of promising new platforms or culture methods that have merely used animal cells so far; they will be considered as well but a transition to using human cells should be considered essential for a further development of those systems.

Even though the systemic clinical picture of DM cannot be reflected by modeling processes occurring in a single organ, breaking the complexity of diabetes down to individual aspects can still provide valuable insights into the integral disease mechanisms. Hence, in the following sections, different in vitro models of T1DM or T2DM key players – namely pancreas, immune system, liver, adipose tissue and skeletal muscle – will be evaluated with respect to their impact on DM research. Further, special emphasis will be placed on platforms featuring co-cultures and multi-organ integration relevant for DM.

3.2.1. Pancreas (endocrine)

B-cells depletion and dysfunction are major factors leading to the development of DM. In T1DM, the precise contribution of genetics and environmental triggers, as well as the defined underlying molecular mechanisms leading to the development of autoimmunity and β-cells destruction are still largely unknown. Therefore, modeling of the endocrine pancreatic function in vitro is of particular interest to deepen our knowledge on the molecular mechanisms involved and to generate patient specific models representative of the disease heterogeneity, which can be use as drug screening platforms for the treatment of diabetes.

3.2.1.1. Cell sources for endocrine pancreas models & challenge of β cells differentiation. The pertinence of in vitro models relies in part on the cell source used, particularly when it comes to the mimicry of human physiology. Primary cells, cell lines and stem cell derived-cells have been employed in in vitro systems. Primary human and animal pancreatic islets are the most extensively used models and are the gold standard for β-cell and diabetic research as they have already provided a considerable amount of molecular, cellular and genetic information [178]. Due to the high variability between patients as well as the tremendous cost generated by islets isolation, the use of cell lines as alternative have been of interest. However to date, only a limited number of human β-cell lines are available, which is in contrast with the numerous rodent-derived insulinoma cell lines such as MIN-6 or INS-1E [179]. Since primary β-cells are mainly non-proliferative in vivo – which is open to debate based on recent studies [180] – many research groups have attempted to overcome replicative senescence while maintaining their functional properties including glucose stimulated insulin secretion. Until recently, the available human cell lines such as CM, TRM-1 or Blox5 were only expressing GLUT2 with a debatable insulin expression and secretion (cf. Skelin et al. [179] for full review). However in 2011, Ravassard and colleagues reported the first human cell lines (EndoC-βH1) based on a multistep process, which lead to functional β-cells capable of secreting insulin in response to glucose challenge [181]. Ravassard et al. transduced human fetal pancreas buds with lentiviral vectors expressing the large T antigen of simian virus 40 (SV40LT) and human telomerase reverse transcriptase (hTERT), before implantation in SCID mice allowing in vivo differentiation and maturation. The immortalizing transgenes SV40LT and hTERT are after the promoter of the rat insulin 2 gene, allowing these specific transformed cells to be later expanded in culture. Subsequently, the next generation of human cell lines (EndoC-βH2 and EndoC-βH3) were derived from the EndoC-βH1 cell line using conditionally immortalized transgenes, which leads to β-cell proliferation arrest resulting in cells highly representative of human primary β-cells [182,183].

An attractive alternative and similarly a major challenge in the field is the generation of mature and functional stem cell derived β-cells [184]. Over the past two decades, advances in technology and methodology have brought us closer to the goal of routinely generating functional glucose responsive human pancreatic β-cells. The first report of pancreatic β-like cells was in 2001, where embryonic bodies were partially glucose-responsive and secreted low levels of insulin [185]. Protocols using embryonic bodies provided the first evidence of the possibility to derive β-like cells from hPSCs, by detecting insulin and showing expression several pancreatic genes including PDX1, transcription factors essential for pancreatic development [186–188]. However, the glycemic control of diabetic mice couldn't been restored upon transplantation of these β-like cells [188]. Protocols using 2D monolayer cultures achieved the direct differentiation of hESCs toward pancreatic endocrine lineage cells by recapitulating different developmental stages [189]. Differentiation protocols were optimized in a large collaborative effort, to increase the yield of insulin positive cells from < 8% C-peptide positive cells [190], to ~15% [191] and in 2009, using for the hiPSCs, up to 25% [192]. However, upon transplantation, only 30% of diabetic mice exhibited an apparent restoration of blood glucose levels for 6 weeks [191]. At this point, the differentiation towards β-cells is

inefficient, generating low percentages of β-like cells together with poly-hormonal cells – endocrine cells co-expressing insulin, glucagon, somatostatin, ghrelin or pancreatic polypeptide – altogether suggesting an immature stage of derived β-cells.

In vivo maturation gained momentum when Shim et al. demonstrated that pancreatic and duodenal homeobox 1 (PDX1) positive endoderm cells can undergo further maturation in vivo after implantation [193]. Subsequent reports revealed that hESC/iPSC-derived endoderm cells have the capacity to mature in vivo into glucose-stimulated insulin secreting β-cells [194,195] with a potential to reverse diabetes in mice models [196–199]. Even though differentiation towards functional mature β-cells was achieved, the pathways promoting maturation remained poorly understood, representing a major drawback for clinical translations and applications. Nevertheless, sophisticated sorting experiments from Kelly et al. identified the progenitor cells population as the source of functional β-like cells after implantation [200]. These progenitors have been characterized as expressing PDX1 TF and homeobox protein NKX6.1 [200]. Based on these findings, Russ et al. hypothesize that generation of polyhormonal endocrine cells in vitro is a result of premature assignment to the endocrine fate [201]. By delaying the use of BMP inhibitors, glucose-responsive β-like cells were successfully generated in vitro (for a complete comparison between protocols see review from Loo et al. [184]) [201]. Vast improvements have been made in the understanding of β-cells differentiation since the first report in 2001. However, the in vitro maturation of β cells progenitors into cells with physiological relevant insulin secretion profiles remains as the main challenge at this point.

3.2.1.2. Conventional endocrine pancreatic in vitro models. Primary isolated human islets retain their glucose-responsiveness and insulin secretive properties in vitro, and therefore have been widely exploited in pharmacological research to test various diabetes related drugs, such as insulin secretion enhancers or inhibitors [202]. Since glucose responsive cells lines (EndoC-βH1-2-3) have only been recently accessible, isolated human islets in culture have been considered the gold standard [178]. However, their utilization for long-term studies is strongly limited due to rapidly occurring loss of function of the ex-vivo islets; typically by 60% within two weeks of continuous culture [203]. During islet isolation the main vasculature is damaged, leaving passive diffusion the sole supply of oxygen and nutriment to the inside of the islets [204]. A variety of groups have aimed at re-engineering primary islets by dissociating the cells and re-assembling them as spheroids of defined sizes – so-called pseudo-islets [205–207]. In a recent study, Zuelig et al. compared intact isolated islets with re-assembled pseudo-islets of human and rat origin and showed that passive diffusion was enough to sustain the required oxygen and nutrient levels in the pseudo-islets due to their small size [206]; indicating an improved long-term culture capacity in vitro.

One alternative to primary islets are cell lines; which have been reported by numerous groups to have a higher stimulation index and insulin secretory capacity when cultured as 3D spheroids, indicating the positive effect of cell-cell contacts [208,209]. Hence, conventional in vitro models have been focused on generating β-cells pseudo-islets via different approaches (comprehensively described in a recent review from Gao et al. [210]). Spontaneous formation or so-called self-assembly is the most straight-forward approach requiring solely low-adherence culture plates. Yet, control over size and composition is very limited, resulting in largely heterogeneous islet populations [211,212]. Microcontact printing is a process based on the localized immobilization of cell-adhesive proteins providing a defined area of contact for the cells and hence enhancing their aggregation [213]. Microwell-based methods rely on self-assembly in miniaturized cell culture wells and are highly attractive in particular for high throughput applications [207,214]. The latter two approaches have been successfully employed to generate large quantities of pseudo-islets with controlled sizes: Hraha et al. designed a microwell-based platform to analyze electrical dynamics (calcium oscillation and propagation) in MIN-6 pseudo-islets as a function of their size [215]. To further improve microwell-based methods, Shinohara et al. developed a polydimethylsiloxane (PDMS)-based platform with improved oxygen permeability compared to traditional polystyrene systems [216].

In an effort to assess the potential of pseudo-islets for drug testing, Amin et al., developed a high-throughput platform to run large-scale compound screens, comparing their previously re-engineered primary human pseudo-islets with pseudo-islets and monolayer culture from rat cells lines [209,217]. They validated their platform using reference drugs and showed that pseudo-islets that exhibit a robust glucose response can provide a valid phenotypic screen for potential diabetic drug candidates [217]. Pseudo-islets have also been utilized in in vitro models to investigate the impact of hypoxia, as hypoxic microenvironments are usually found in vivo after a standard transplantation of islets or cells. Using the conditionally immortalized human cell line EndoC-βH3, Lecomte et al. assessed the impact of hypoxia on gene expression, as well as the role of hypoxia inducible factor (HIF). The EndoC-βH3 cell line is opening new pathways for β-cell research by excising the immortalizing transgenes at any time point, thereby bringing the cell´s phenotype closer to primary β-cells. In a recent publication the role of a specific long-non-coding RNA (lncRNA) network was uncovered using EndoC-βH3 cells as model [218]: The authors showed that a specific set of lncRNAs frequently regulate genes associated with islets enhancers crucial for islet function [218]. In addition, they showed that the lncRNA PLUTO is controlling PDX1, which was a highly interesting finding considering that PLUTO and PDX1 are downregulated in T2DM isolated islets [218].

To promote long-term culture while preserving cell functionality and viability, multiple research groups developed engineered 3D microenvironment that recapitulate the native in vivo microenvironments. Important characteristics, thereby, are the mechanical and biochemical properties of the native environment, which were tuned using hydrogels of different stiffness and by providing specific ECM binding sites encouraging cell-ECM and cell-cell interactions. Essential ECM proteins, such as laminin, collagen, fibronectin or E-cadherin, have been reported to maintain islets morphology as well as functionality and to support long-term in vitro culture [219–222]. Szebeni et al. used a collagen I based hydrogel within a *Real Architecture For 3D Tissue* (RAFT™) culture system to maintain primary rat islets in long-term culture [220]. Similarly, Li et al. developed an 3D perfused islet model suitable for diabetic drug studies [223]. Their system is composed of multiple, parallel perfused microbioreactor systems allowing the culture of primary islets in TissueFlex®. GLP-1 stimulated the 3D in vitro models in a dose-dependent monophasic manner in contrast to 2D monolayer models, which did not show a clear dose-response [223]. Besides providing physiological microenvironment, hydrogels have been also employed for the encapsulation of 3D islets prior to transplantation as summarized in detail by Gorka et al. [224]. Knowledge and experience gained in those studies, including insights into the various biomaterials, could also be highly interesting for the development of in vitro models for drug screening applications or basic research.

3.2.1.3. IPS-derived β-cell-based in vitro models. Harboring the same genetic information as patients from whom they have been generated, hiPSCs have been of particular interest to generate specific diseased models that recapitulate the in vivo disease phenotype. Shang et al., for instance, developed a stem cell-based model of Wolfram syndrome; a disease caused by mutation of WFS1 and characterized by insulin dependent diabetes, optic atrophy and deafness [225]. In this study, patient-derived β-cells exhibited a lower level of insulin and an increased activity of three major UPR pathways. The addition of chaperone 4PBA reduced the level of UPR signaling pathway molecules and increased the insulin content. This illustrates the great potential of disease models based on stem cell derived cells. Similarly, hiPSC in vitro

models have been used to investigate molecular pathways involved in the Maturity Onset Diabetes of the Young (MODY) [226–228], which is a monogenic form of diabetes arising from one or more mutations in autosomal dominant genes [229]. Yabe et al. revealed that mRNAs from MODY5-hiPSCs had a mutation leading to a premature termination codon (PTC) [226]. Moreover, MODY mRNAs with PTC were degraded by nonsense-mediated mRNA decay during differentiation into pancreatic β-cells. In another study, Teo et al. showed that MODY5 caused a compensatory increase in several pancreatic transcription factors and a decrease in the expression of PAX6, an important gene for β-cell function [228].

3.2.1.4. Pancreas-on-a-chip. The development of pancreas-on-a-chip systems is currently still in its infancy. Most introduced systems so far focus on short-term culture and functionality screening of pancreatic islets [230–233], in contrast to OoCs for other tissues that try to recapitulate long-term functionality of tissues for disease modeling and drug testing. The underlying motivation thereby is the rapid assessment of the islets' functionality during the process of islet transplantation therapy. A precise knowledge of the quality of a donor's pancreatic tissue is of upmost importance for better transplantation decisions. One of the first microfluidic devices integrating and perfusing islets was introduced by Mohammed et al. in 2009 [230]: using both murine and human islets, the platform enabled multimodal functionality assessment by fluorescence imaging of intracellular calcium, measurement of mitochondrial membrane potential as well as quantification of secreted insulin (Fig. 4A) [230]. Other perfused well platforms were developed by the Easley research group to assess dynamics of hormone secretion from cells, including insulin secretion by islets [231,232]: the basic concept behind their systems is a macro-to-micro interfacing allowing a temporally resolved examination of tissues. An additional integration of a microfluidic input/output multiplexer (μMUX) implements an accurate control over nutrient input and hormone output [231], while 3D-printing of the interface templates accelerated prototyping [232]. In a different approach, islets were traped in micro-traps and surface tension gradients harnessed to passively pump liquids enabling the profiling of insulin kinetics [233]. Trapping pseudo-islets in micro-wells, a recently introduced glass-made microfluidic platform allowed for the evaluation of the coupling between a stimulus and its associated secretion in pancreatic islets [234]. Similarly, a microphysiological analysis platform (MAP) that enabled both the formation as well as analysis of human cell line based pseudo-islets was reported by Lee et al. [235].

3.2.1.5. Summary and perspectives with respect to DM research. In the last five years, the first human β-cells lines were successfully generated, facilitating the access to an adequate cell source, as well as dramatically reducing the costs generated by islets isolation. Extensive progress has been made in terms of the differentiation of stem cells towards pancreatic β-cells, which is especially interesting with regards to patient- and disease-specific hiPSCs. Besides that, substantial evidences from various research groups have reported the benefit of using pseudo-islets from cell lines or from primary cells compared to 2D culture and microfluidic systems have been developed that enable a rapid assessment of islets/pseudo-islets vitality and functionality. Although the current microfluidic platforms have mainly been designed for islet quality assessment for subsequent in vivo implantation, these recent progresses are highly encouraging and have provided the scientific community a solid base for further improvements. By combining the emerging hiPSC technology with microphysiological systems (MPS), long-term

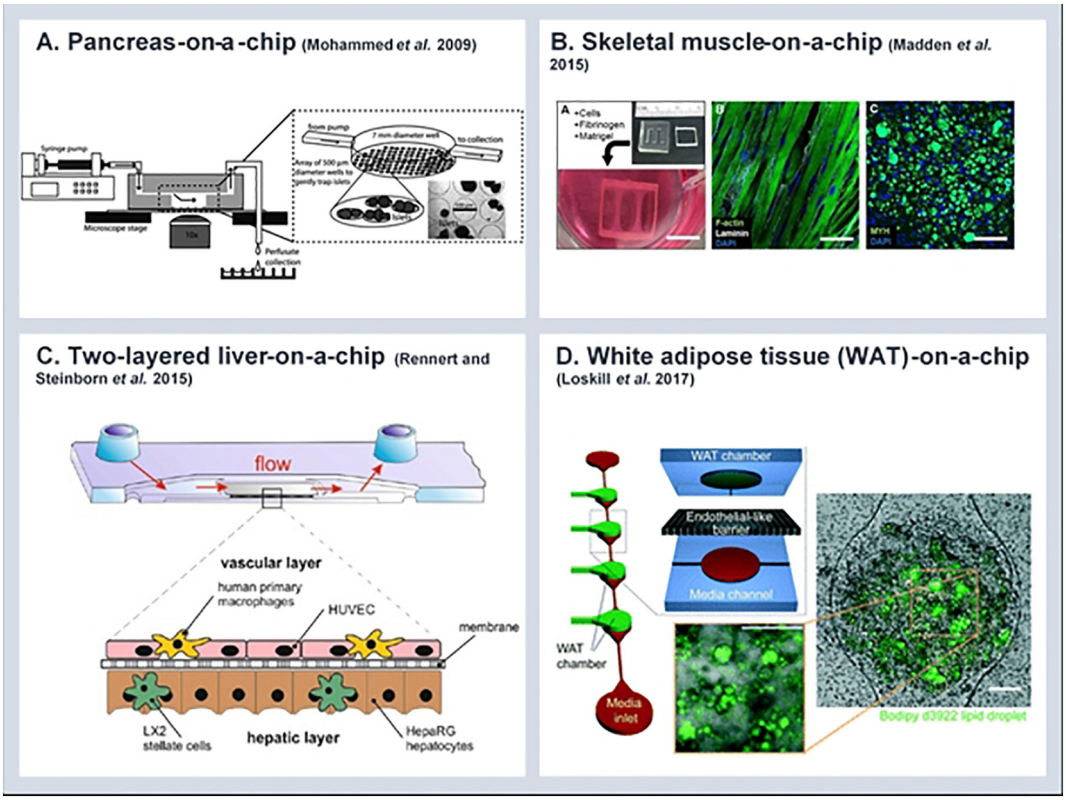

Fig. 4. Single-organ chips with a future potential to impact DM research. A. One of the first pancreas-on-a-chip systems targeting rapid assessment of human islet functionality. Reproduced from [230] with permission of The Royal Society of Chemistry. B. Human skeletal muscle microfluidic system enabling myofiber alignment as reflected by F-actin- and myosin heavy chain (MYH)-staining. Reproduced from reference [312] (CC BY 4.0). C. Human two-layered liver-on-a-chip system mimicking the hepatic sinusoidal structure [264] [174]. Reprinted from Biomaterials, 71 (2015), K. Rennert and S. Steinborn et al., A microfluidically perfused three dimensional human liver model, 119–131, Copyright (2015), with permission from Elsevier. D. WAT-on-a-chip accommodating functional murine adipocytes as revealed by a staining of intracellular lipid droplets. Reproduced from [293] with permission of The Royal Society of Chemistry.

culture of patient specific tissues could provide reliable platforms for diabetes research and drug screening.

3.2.2. Immune system

The immune system plays a significant role in DM, particularly with T1DM, where β-cells depletion arises from an autoimmune response [236]. Despite considerable research, the mechanisms regulating the immune response on β-cells remain partially unknown. Although diabetes research has provided considerable amount of data and evidences, the causes and mechanisms involved in the autoimmune attack of β-cells remain partially unknown. For instance, impairment in negative selection during pancreatic growth plays an important role in the development of auto-reactive lymphocytes, occurring through a complex combination of genetics and environmental factors. Modelling an immune response in its entirety is highly complex as it employs a variety of specialized cells, molecules, tissues and organs in a synchronized cascade of events with the aim to fight endogenous dysfunction or exogenous bodies. Modeling the complex immune system response in vitro is extremely difficult. Currently, there is no in vitro system modelling autoimmune interactions for diabetes research. Nevertheless, considerable progress has enabled the modelling of parts of the immune cascade in different physiological and pathological scenarios in vitro. For instance, T-cell and neutrophil migration behavior in response to different chemical gradient profiles as well as migration patterns of T-cells, macrophages and dendritic cells in response to bacteria have been investigated in vitro [237]. Moreover, the adhesion of T-cells to and the rolling of neutrophils along artificial surfaces have been modelled in microfluidic channels [238,239]. First steps towards the interaction of immune cells with other tissues have also been achieved by modelling the transmigration of T-cells, neutrophils and monocyte through an endothelial monolayer in vitro [240,241] and by integrating immune components into OoC systems, generating inflammation models to mimic pathological situations in a lung-on-a-chip [242], a gut-on-a-chip [243] and a liver-on-a-chip model [244]. The principle similarities of these OoC systems are (1) a porous membrane delimiting an epithelium (airway, intestinal or hepatic) from a perfusion compartment; (2) the perfusion with primary peripheral blood mononuclear cells (PMBCs) isolated from patient blood; and (3) the recruitment of immune cells via cytokine signaling.

In these examples, hindering direct cell-cell contact between immune cells and epithelial cells is crucial as they originate from different individuals. This is an essential aspect to consider when it comes to model autoimmunity in vitro, as it requires direct interaction between APCs and auto-reactive lymphocytes. In order to model an autoimmune reaction without initiating an immune rejection cascade, the two cell types should originate from the same individual, which could be possible using iPSC technology. Although modelling the autoimmune attack of β-cells in vitro is highly ambitious, it would be highly valuable for T1DM research by providing a platform to study molecular mechanisms in the interactions of β-cells and immune cells.

3.2.3. Liver

The liver is the largest internal organ of the human body and plays a vital part in governing digestion and metabolism. In patients with T2DM, the liver is one of the first organs to become insulin resistant as a consequence of chronic excess dietary energy. In the cascade of T2DM events, the liver responds to chronic systemic hyperglycemia by even further increasing *de novo* production of glucose. Besides its essential relevance for metabolism, the liver is of utmost importance for detoxifying and eliminating fat-soluble toxins from the body. Consequently, the development of conventional hepatic in vitro models and liver-on-a-chip platforms is strongly attracting pharmacological and toxicological research.

3.2.3.1. Cell sources for hepatic in vitro models. The architecture of the liver is featured by complexly structured, zonal lobules. They are comprised of parenchymal cells, i.e. hepatocytes (60%), and non-parenchymal cells (NPCs) (40%), such as stellate cells, Kupffer cells and endothelial cells [245]. Although hepatocytes usually are the focal point in in vitro models aiming to reflect either a disease mechanism or a compound's effect on the liver, NPCs should not be overlooked due to their contribution to a secondary response to hepatotoxicity [245].

To date, there are three main sources for hepatocytes for in vitro models: primary human hepatocytes (PHHs), liver cell lines, and pluripotent stem cells. As a result of their close resemblance to in vivo functionality, PHHs currently are the gold standard for hepatic culture models [246]. Yet, due to a decreasing functionality in long-term culture [247] and the limited accessibility of PHHs, other sources of hepatic cells had to be investigated. Liver cell lines are either obtained from tumor tissue or by genetic engineering of PHHs; they are widely used because of their low costs and availability but their high proliferative potential is accompanied by functional losses. HepG2 and HepaRG, the presumably most prominent hepatic cell lines, are both generated from human hepatoma [246]. HepG2 is best used to model hepatic cancer and associated treatment strategies [246], but it is not suited for studying hepatic biotransformation due to atypical quantities of drug metabolism enzymes and deficient metabolic activities [248]. In contrast to HepG2, the HepaRG cell line is an alternative to PHHs in the context of metabolic or toxicological studies; because of a higher grade of differentiation, HepaRG cells retain more hepatic metabolic functions, including drug-metabolizing cytochrome P450 (CYP) enzyme activities [246,249]. Another alternative to PHHs are human pluripotent stem cells differentiated along the hepatic lineage. Yet, the generation of hepatocytes derived from hiPSCs – referred to as 'hiPSC-derived hepatocyte-like cells (hiPSC-Heps)' – is extremely challenging: even though there are differentiation protocols, which have proven to be efficient and reproducible, hiPSC-Heps' functionality still rather resembles immature or even fetal hepatocytes. One of the most confining hurdles resulting from hiPSC-Heps' immaturity is the extreme decrease in the activity of a significant number of CYPs. As in vivo differentiation from fetal to adult hepatocytes is not fully understood yet, further knowledge about this developmental step is necessary to improve the progress towards obtaining adult hiPSC-Heps [250,251].

In sum, balancing the pros and cons when choosing the optimal source of hepatic cells for a specific application is crucial because the cell source determines the model's relevance and its predictive efficiency [248].

3.2.3.2. Conventional hepatic in vitro models. To mimic liver disease mechanisms or hepatic responses to novel drug candidates, engineered human liver models should recapitulate the in vivo situation of the aspect to be represented as close as possible while minimizing the model's complexity. Existing types of human liver models are clearly distinguishable from each other by the extent to which they mimic in vivo structure and liver functions.

A very basic approach towards a physiologically-relevant human hepatic in vitro model is the use of acellular enzymatic platforms; by analyzing liver microsomes containing CYPs in high-throughput platforms, information on a drug candidate's mode of action in the liver can be retrieved [252,253]. Precision-cut liver slices are also widely used as model systems. They preserve the natural liver structure, contain all cell types, and can even be cryopreserved [254,255]. However, the limited access to human liver tissue and the low throughput character strongly limits the applicability of these systems as drug testing platforms. As with PHHs, another drawback of *ex vivo*-cultured human hepatic tissue is a rapid loss of functionality [247]. To prevent this effect, micropatterning and 3D modeling have been proven helpful [247].

An interesting hepatic in vitro model was established by making use of micropatterning [247,256–259]: first, compared to conventional 2D monolayer culturing, in vitro culture conditions of PHHs were found to be improved in micropatterned co-cultures (MPCCs) of

cryopreserved PHHs and stromal fibroblasts. Further, the same microscale liver system was investigated with regard to responsiveness to fasting and feeding states; by introducing insulin and glucagon to the system, state-dependent glycogen lysis and storage was revealed. Moreover, hepatic MPCC gluconeogenesis was found to be modulated by an administration of metformin, and in response to hyperglycemia, intracellular lipids inside PHHs were raised [247]. In a subsequent study, the MPCCs were exposed to long-term pathophysiological glucose levels, i.e. either hypo- or hyperglycemia. Already within six days of culture under constant hyperglycemic conditions (25 mM glucose), PHHs showed an onset of insulin resistance. The accumulation of intracellular ectopic lipids was found to be raised already for lower glucose levels (12.5 mM glucose) [256]. Aside from modeling the development of hepatic insulin resistance – highly relevant for T2DM – as well as hormone- and drug-mediated glucose metabolism, the MPCC technology was also applied to examine hiPSC-differentiation. Several studies found that the micropatterned co-culture enhanced maturity and longevity of hiPSC-Heps [258,259].

3.2.3.3. Liver-on-a-chip. On account of the liver's high relevance for drug testing and toxicology studies, hepatic tissue ranks among the most researched organs in the field of OoCs. Up until now, a significant number of liver MPSs have been introduced each featuring different strategies for recapitulating both in vivo hepatic function and architecture [260–269]. Due to the broad range of liver-on-a-chip systems, only some platforms will be presented exemplarily in this review.

An important aspect to keep in mind when designing a liver-on-a-chip is the fact that multiple hepatic cell types are involved in a response to liver injury and hepatotoxicity. Therefore, from a biological perspective, it is necessary to implement co-culture hepatic models. From a structural perspective, a resemblance to in vivo liver architecture and the in-chip flow conditions play an important part. One example of a liver MPS integrating multiple cell types in a perfused environment was presented by Esch et al. [265]: The metabolic functionality of a human 3D microfluidic culture containing hepatocytes, fibroblasts, stellate cells and Kupffer cells was shown to be improved compared to static culture conditions.

Another liver MPS approach constructed perfused 3D spheroidal liver models [260]: Bhise et al. 3D-printed human hepatic HepG2/C3A spheroids in a photo-crosslinkable hydrogel into the culture chamber of a bioreactor. Apart from the maintenance of long-term hepatic functionality, this study is interesting from a technical point of view as well; hepatic spheroids are printed directly into the microfluidic device, which is then assembled around the printed tissue. Unlike most other microfluidic platforms, it can be easily disassembled allowing simple access to the hepatic tissue after the experiment [260].

A concept which additionally recapitulates the in vivo structure of the liver was established by Mosig and colleagues [263,264]: their platform integrates a two-layered tissue which is closely mimicking the morphology of a liver sinusoid. While the hepatic layer of the device features a co-culture of hepatocytes and stellate cells, the vascular layer consists of non-parenchymal cells, endothelial cells as well as tissue macrophages (Fig. 4C). Via integrated sensors based on luminescence, the liver equivalent's oxygen consumption can be measured online [264]. In the further development of this liver-on-a-chip platform, the MPS was transformed into a disease model by inducing an inflammation-associated dysfunction of the liver. This condition was triggered by the release of pro- and anti-inflammatory cytokines by means of the administration of toll-like receptor (TLR) agonists to the liver platform. When monocytes were integrated into the inflamed liver-chip, the tissue response to the TLR agonists was found to be attenuated [263].

On account of the liver's relevance for disease modeling and the evaluation of patient-specific drug responses, the combination of OoC and hiPSC technology is advancing with great strides. Schepers et al. have developed a microfluidic device incorporating hiPSC-derived hepatocytes [270]: 3D aggregates of hiPSC-derived cells were encapsulated either individually or in co-culture during their differentiation towards hepatocytes and were examined with respect to stable albumin production and inducible CYP activity. Both analyses confirmed the hepatic tissue inside the chips to be viable and functional. However, unlike the chips mimicking the architecture of a hepatic lobule, this chip features C trap structures to hold the formed microtissues in place [270]. Based on a different approach, Banaeiyan et al. established a liver MPS featuring hexagonal structures of liver-lobuli [266]. In comparison to other organ-on-a-chip platforms, this system is a large-scale model (with one hexagonal structure being in the range of several millimeters) also giving rise to the denomination as "very large-scale liver-lobule (VLSLL)-on-a-chip device". To supply nutrients to the hexagonal lobule-like chambers, the media channels were designed to mimic the fenestrated endothelial cells of the liver, based on a concept initially introduced in a liver chip model by Lee and colleagues [262]. Inside the system, hiPSC-derived hepatocytes formed bile canaliculi and maintained their functionality for three weeks as validated by assessing viability, albumin secretion and urea synthesis [266].

3.2.3.4. Summary and perspectives with respect to DM research. The research and progress in the development of in vitro liver models doubtlessly profits from the liver's relevance for pharmacological and toxicological studies. There is a broad, well explored choice of hepatocyte sources ranging from PHHs over hepatic cell lines to hiPSC-derived liver cells. Diabetic research will profit most from the use of hiPSC-derived hepatocytes as it enables the generation of patient-specific cells and consequently patient-specific liver responses. Yet, obtaining hiPSC-Heps is still challenging and held back by their immaturity. In addition, research on hiPSC-derived hepatic cells focuses mostly on hepatocytes only; however, NPCs are also required to generate physiologically functional liver models. Even though there is no standalone diabetic liver-on-a-chip platform yet, the advances made are promising. A combination of the presented hepatic in vitro models reflecting the onset of insulin resistance in the liver [247,256–259] and an adequate microfluidic environment would constitute an outstanding platform for studying hepatic insulin resistance as a target for T2DM treatment strategies. Moreover, as discussed in Section 3.2.6, liver MPS are fundamental building blocks for a multi-organ platform for reflecting the systemic character of T2DM.

3.2.4. Adipose tissue

WAT is one of the key players in the development and manifestation of T2DM. According to its physiological function, WAT is supposed to clear glucose from the blood stream and store the associated energy in the form of lipids. However, this mechanism is disrupted as a result of insulin resistance and enhanced lipolysis in WAT which are both caused by macrophage infiltration in response to excess dietary energy as well as insulin resistance in liver and skeletal muscle. Aside from WAT's relevance for finding new diabetes treatment options via modeling of inflammation or insulin resistance, adipose tissue is also a target in the prevention of T2DM; a future therapy option to counteract obesity might be the expansion of thermogenically active brown adipose tissue (BAT). This can be realized by gene therapy, pharmacologic induction of WAT-to-BAT transition – a method also referred to as 'browning' – or differentiation of adipose-derived stem cells into beige adipose tissue [271].

3.2.4.1. Cell sources for adipose in vitro models. For the generation of adipose in vitro models, multiple resources for obtaining adipocytes are available. First of all, the use of primary cells is more the custom in the field of adipose tissue engineering compared to other tissues due to the relatively uncomplicated accessibility to primary human mature adipocytes; adipose tissue samples occur as a by-product of plastic surgery (subcutaneous adipose tissue) or visceral surgery (visceral adipose tissue) [272,273]. Moreover, primary mature adipocytes retain patient-

specific information which is especially important in terms of reflecting characteristics of adipose-related diseases such as the hypertrophy in obesity, for instance [274]. However, the handling of mature adipocytes poses a major challenge. Firstly, they are terminally differentiated and can neither be expanded nor cryopreserved [275,276]. Therefore, other sources for adipocytes are available and thoroughly explored: in vitro differentiation of adipogenic progenitor cells (i.e. either preadipocytes or multipotent stem cell lines) or hiPSCs is of special interest due to their capacity to be expanded and cryopreserved while retaining the characteristic fat depot and patient-specific information [276]. The use of adipose cell lines, however, has two major limitations: first, they contain many small intracellular lipid droplets instead of the in vivo-occurring single large vacuole. Second, the secretome of adipose-associated hormones, adipokines, differs strongly from in vivo [277]. A further drawback of using preadipocytes is their limited capacity to differentiate with increasing number of passages [278]. With regard to hiPSC-WAT, the progress on generating hiPSC-derived white adipocytes is slowed down by a current focus on obtaining brown or beige adipocytes, rather than white adipocytes. This is due BAT's and beige adipose tissue's promising actions as a therapeutic target for obesity [279–281].

Especially interesting in the context of DM is a recent study evaluating the adipogenic differentiation potential of MSCs derived from T2DM patients: interestingly, diabetic MSCs seemed to lose their capacity to differentiate into mature and functional adipocytes as reflected by differences in their genetic profile compared to healthy control MSCs. This dysfunction of MSC differentiation might be caused by adipose tissue inflammation and was found to be accompanied by adipocyte hypertrophy and diminished adipose tissue turnover [282].

In general, due to their unique properties, including buoyancy due to their lipid depots as well as extreme fragility and possible dedifferentiation under inadequate in vitro conditions [283], models using adipocytes require sophisticated strategies to generate physiologically relevant in vitro WAT.

3.2.4.2. Conventional adipose in vitro models. The range of adipose in vitro models is broad; however, adipose tissue engineering often focusses on soft tissue replacements for regenerative medicine [284,285], which are not suitable for metabolic studies or compound testing. Solely a few examples so far have targeted adipose tissue micro-platforms or modeled adipose tissue inflammation, i.e. a hallmark of T2DM.

One approach is the utilization of 3D silk protein scaffolds to accommodate *ex vivo* white adipose tissue [286–288]. In a further step, the 3D silk scaffolds were integrated into 2D and 3D macroscopic perfusion systems for long term culture of adipose tissue; both 2D and 3D systems were successful in modeling adipogenesis of human primary preadipocytes. Yet, as a consequence of a higher mechanical support, 3D systems were found to be more robust than 2D setups as revealed by greater lipogenic and lipolytic activities [289].

In order to model adipose tissue inflammation, Turner et al. introduced a spheroid adipogenesis model to investigate the influence of an inflammatory microenvironment. Murine 3T3-L1 preadipocytes were exposed to pro-inflammatory stimuli during differentiation by modulating cell maturation via elevated levels of fatty acids followed by an exposure to TNF-α. Exposed adipose tissue showed a reduced metabolic function characterized by enhanced lipolysis and reduced expression of peroxisome proliferator-activated receptor gamma (PPAR-γ), an important factor involved in adipogenic differentiation and accumulation of triglycerides [290]. Zagotta et al. proposed a human adipose in vitro inflammation model to evaluate the effect of resveratrol, a drug candidate to treat obesity. They found that in obesity-induced inflamed adipose tissue the drug reduced the production of inflammation-associated cytokines [291].

3.2.4.3. Adipose tissue-on-a-chip. The development of adipose tissue MPS also caught attention lately. Some OoC-platforms manifested to be especially advantageous because of the microfluidic environment designed to protected integrated fragile adipocytes from fluid flow and shear forces. One of the first attempts to integrate adipose tissue in a microfluidic platform was published by Viravaidya and Shuler in 2004: besides a lung and a liver compartment, their "microscale cell culture analog (μCCA)" device featured a chamber accommodating differentiated 3T3-L1 murine adipocytes to study bioaccumulation of toxins [292]. Another promising tool for drug testing and disease modeling that integrates WAT was only recently introduced by Loskill et al.: in-chip adipogenic differentiation of 3T3-L1 murine adipocytes resulted in a mature adipose tissue with physiologically functional lipid metabolism which was stable for multiple weeks. The WAT-chip architecture was, thereby, specifically adapted to accommodate adipocytes and shield them from shear forces by featuring a tissue chamber and a media channel system (Fig. 4D) [293].

While the previously discussed system mainly focused on the generation of a functional adipose tissue microphysiological platform for drug testing and disease modeling, another two-compartment adipose tissue device was developed to emulate insulin-dependent glucose uptake in adipocytes [294]: 3T3-L1 murine preadipocytes were cultured as 'adipo-spheroids' inside a perfusable type I collagen-filled chamber. Insulin-supplemented media was added via a "blood compartment" and an insulin-triggered glucose uptake was shown. Apart from investigating the functionality of the adipose glucose metabolism, this study especially highlights another important aspect of diabetes in vitro models, namely the significance of relative scaling of organ-on-a-chip systems: if the adipose tissue and the "blood volume" were not scaled properly relative to each other, the systems would constantly be exposed to hypo- or hyperglycemia [294].

In addition to those OoC platforms featuring tissues in a microphysiological environment, there are several microfluidic adipose tissue platforms that are based on microfluidic interfaces. Those devices concentrate on time-resolved sampling of tissue secretions and thereby on a quantification of endocrine tissue functionality: in the case of adipose tissue, glycerol [232,295] as well as adipokine secretion dynamics [296] were determined.

3.2.4.4. Summary and perspectives with respect to DM research. For DM research, adipose tissue is of interest in two important aspects: On the one hand, adipose tissue is a prominent site of tissue insulin resistance and chronic inflammation making it a main target for mechanistic and therapeutic research. On the other hand, a conversion of white adipocytes to beige adipocytes could help prevent metabolic disorders like obesity. Cell sources available for human adipose in vitro models range from primary mature adipocytes over preadipocytes and multipotent stem cells to hiPSC-derived adipocytes. However, unique characteristics of adipocytes, including buoyancy and extreme fragility, necessitate elaborate culturing and encapsulation techniques. The majority of tissue engineered in vitro models concentrates on establishing large tissues applicable for soft tissue regeneration; only few models investigated adipose-related disease mechanisms in a micro-scale. Only recently, the OoC technology began to address the challenge of developing adipose tissue MPS. Even though most systems do not integrate human but murine cells, the platforms present a solid foundation for future human adipose tissue-on-a-chip systems with a promising potential in diabetes research.

3.2.5. Skeletal muscle

Skeletal muscle is the first tissue to develop an insulin resistance in response to ectopic lipid accumulation caused by excess dietary energy. By unloading the burden of elevated blood glucose levels onto other tissues, especially onto the liver, the insulin resistance spreads out onto those tissues as well. This mechanism is an integral part of T2DM. Therefore, investigating the mechanisms behind skeletal muscle insulin

resistance and its prevention, or even reversion, is highly interesting within the scope of diabetes research.

3.2.5.1. Cell sources for skeletal muscle in vitro models. Similar to adipose tissue engineering, the main motivation behind skeletal muscle tissue engineering so far has been to develop muscle grafts used for reconstructive surgery. Owing to the high cell numbers required to generate large muscle grafts, the cell sources exploited to engineer new functional skeletal muscle tissues need to be readily expandable [297]. Moreover, skeletal muscle precursor cells must have the potential to be differentiated from mononucleated cells into densely packed, multinucleated myofibers, which are capable of maintaining muscular function, i.e. uniaxial contraction [298,299]. One commonly used cell source are primary satellite cells; their myogenic differentiation resembles the in vivo situation much better compared to programming immortal cell lines, as e.g. C2C12 (mouse) or L6 (rat) cells [298]. Further cell types featuring a myogenic potential include MSCs [300], embryonic stem cells [301], myoblasts [302].

Moreover, in recent years efficient and robust protocols to direct hiPSC differentiation along the myogenic lineage have been introduced [303–306]. However, as it is often the case for hiPSC technology, the maturation state of these hiPSC -derived muscle cells remains unclear. In addition, the contractile function of hiPSC-derived myofibers remains to be validated [299]. Interestingly, hiPSC-based myotube generation already enabled the modeling of skeletal muscle insulin resistance: Iovino et al. compared healthy hiPSC-myotubes to hiPSC-myotubes derived from individuals suffering from Donohue syndrome, i.e. a severe disorder with monogenic mutations in the insulin receptor (IR-Mut) [307]. IR-Mut hiPSC-derived myotubes were found to feature deficient insulin signaling and glucose uptake, erroneous accumulation of lipids, and defective insulin-regulated gene expression.

3.2.5.2. Conventional skeletal muscle in vitro models. Skeletal muscle is a highly specialized tissue, and besides a complex and robust, anisotropic functional 3D architecture, it requires a coupled electrical stimulation capacity along with a mechanical contractibility [308]. In addition to an adequate cell type used for the in vitro model, skeletal muscle tissue engineering demands an elaborate frameworks to present a physiologcial microenvironment featuring anisotropic and elastic cues as well as anchoring properties [299]. Hence, skeletal muscle tissue engineering is closely linked to the research area of biomaterials [297,309], and the combination of these two fields already yielded a variety of skeletal muscle in vitro models which were reviewed by Qazi et al. [297]. Moreover, because of the appeal as a therapy for volumetric muscle defects, vascularization of these large muscle constructs holds another priority within the scope of skeletal muscle tissue engineering [310,311]. Yet, beside the progress in developing skeletal muscle in vitro models, the recapitulation of in vivo organization and function is still limited in conventional in vitro skeletal muscle models [312] resulting in inadequate states of physiological and functional maturity in comparison to intact muscle [299].

3.2.5.3. Skeletal muscle-on-a-chip. Despite skeletal muscle being extremely relevant for diseases with a great impact on society, such as diabetes as well as dystrophic disorders and neuromuscular diseases, OoC systems of skeletal muscle have not nearly as much addresses as in the case of liver or cardiac tissues, for instance. Only recently, the first skeletal muscle-on-a-chip platforms were introduced. The first functional microfluidic model of human skeletal muscle was developed by Madden et al. (Fig. 4B) [312]. Chemically and electrically responsive muscle tissue, referred to as 'myobundles', was engineered by isolating, expanding and differentiating primary myogenic cells. Non-invasive monitoring of muscular function and drug reaction was ensured by measurement of contractile forces and GCaMP6-reported calcium responses. Exemplarily focusing on muscle-related disorders, the applicability of the system as a drug testing device was confirmed via administering lovastatin and cerivastatin. These two drugs are commonly used to prevent coronary artery disease; as a side effect, however, statins can entail myopathic weakness. In form of hypertrophy and toxic myopathy, this effect was detected in the skeletal muscle MPS as well [312]. Other skeletal muscle-on-a-chip platforms were established but, in contrast to the previously discussed system, rely on the C2C12 murine myoblast cell line [313–315]. In a platform established by Argawal et al., cardiotoxin, a substance commonly used to induce damage to skeletal muscle by evoking myotube depolarization as well as rupture of the cytoskeleton, was applied to generate an injury model, whereby a dose-dependent response could be demonstrated [313].

3.2.5.4. Summary and perspectives with respect to DM research. Research combining diabetes and in vitro skeletal muscle platforms is still at a very early stage, and not many studies relate the T2DM-associated insulin resistance to disease modeling in skeletal muscle. Still, the derivation of diabetic hiPSC-derived myotubes exhibiting a severe form of insulin resistance has already been successful. A combination with the skeletal muscle-on-a-chip platforms, which so far mainly focus on system validation and mechanical properties of muscle, would present a milestone towards powerful tools for researching the onset of insulin resistance as well as associated therapy options. Yet, even though the development of in vitro skeletal muscle models is advancing, there are still limitations hampering a faster progress. One of the challenges is the maturity of in vitro skeletal muscle systems: especially hiPSC-derived myobundles cannot recapitulate muscle contractility yet and their degree of maturity remains to be demonstrated.

3.2.6. Multi-organ approaches towards modeling DM

Inter-organ communication plays an important role in human metabolism. Even though recapitulating individual organ-associated aspects of DM can provide valuable insights into respective facets of the disease, conventional single-organ in vitro models fall short on mimicking paracrine or endocrine inter-organ signaling. The systemic and heterogeneous character of diabetes calls for models on a multi-organ level. The following section reviews microfluidic-based in vitro models featuring various inter-organ connections with a high relevance for DM.

3.2.6.1. Multi-organ in vitro models targeting DM research. Despite the difficulties accompanying the development of an adequate DM in vitro model, some research groups ventured to address this challenge in recent years. One such model – an 'endocrine system-on-a-chip for a diabetes treatment model' – was introduced by Nguyen et al. in 2017 [316]. In order to screen diabetic drugs, the platform features an intestinal-pancreatic co-culture: murine L-cells producing GLP-1 and rat pancreatic β-cells producing insulin were integrated into the 3D microfluidic channel-based cell culture system (3D-μFCC) which was originally developed by Toh et al. [317]. By compiling glucose-dependent dynamic profiles of diabetes-associated hormones, this platform holds future promise to screen potential drugs for the treatment of T1DM as well as T2DM (e.g. GLP-1 analogs, natural insulin or GLP-1 stimulants) [316]. However, this system is confined to the interplay between the pancreas and the intestine only, and an integration of further organs, such as the liver, would be required to more precisely reflect DM's heterogeneity. Moreover, the integrated tissues originate from rodent cell lines; thus, a transition of this model towards using human cells, possibly even patient-specific hiPSCs, is considered as indispensable in order to enhance the physiological relevance of the platform.

A first approach to model T2DM in a multi-organ microfluidic platform was published in late 2017 by Bauer et al. [318] (Fig. 5A.): by culturing pancreatic islets and liver spheroids in a two-organ chip, the interaction was investigated based on insulin and glucose monitoring. Human pancreatic islets were obtained commercially (3D InSight™ pancreatic islet microtissues, InSphero AG, Switzerland) while liver spheroids were formed from HepaRG cells. The functionality of this

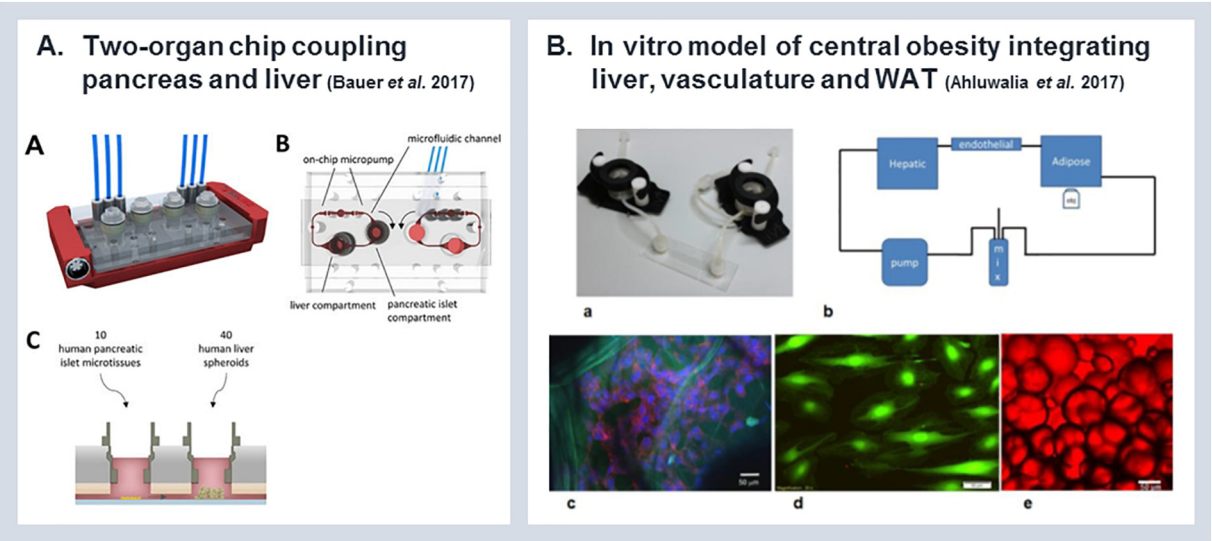

Fig. 5. Multi-organ approaches towards modeling DM. A. A microphysiological two-organ chip coupling human pancreatic islet and human liver spheroid compartments as a future model of T2DM. Reproduced from reference [318] (CC BY 4.0). B. An in vitro model of central obesity featuring human hepatic, vascular and adipose compartments capable of reflecting systemic and vascular inflammation. Reproduced form reference [273] (CC BY 4.0).

system as well as the extent to which it could enable pancreas-liver cross-talk was reflected by a functional in vivo-like homeostatic feedback loop: In response to a glucose load, in-chip islets produced insulin which, in turn, promoted glucose uptake by the liver spheroids; yet, when insulin levels were low, glucose was not consumed by the liver compartment. Moreover, even a simulation of a glucose tolerance test (GTT) as used in humans to diagnose DM resembled the in vivo situation: glucose concentration was raised in the culture medium which, via increased pancreatic insulin secretion, accelerated hepatic glucose uptake. Subsequent to the glucose load, the coupled two-organ platform returned to a fasting glucose state – a mode which was not observed for single cultures. However, Bauer et al. also reported slower kinetics in glucose disposal compared to the in vivo situation which could be attributed to an absence of other glucose-consuming tissues as muscle or adipose tissue [318]. Apart from the integration of further T2DM-associated organs, primarily the hepatic cell source, a hepatoma cell line, should be reconsidered; prospectively, a personalization of the system by using hiPSCs would have a huge impact on more in-depth research of DM therapy options.

3.2.6.2. Multi-organ in vitro models with potential future impact on DM research. Besides the previously discussed multi-organ models directly addressing DM, a significant number of in vitro models implicitly touches on DM-associated facets like human metabolism or DM key organs. Even though those models would require distinctive modifications with regard to DM, they hold potential to significantly contribute to research in this area.

On part of modeling human metabolism, a metabolic in vitro 3-way network of hepatocytes, adipose tissue and endothelial cells based on a multi-compartment bioreactor was established [319]. To assess crosstalk and each organ's individual contributions to visceral energetic substrate metabolism, the tissue network was exposed to normo- and hyperglycemic conditions. Under physiological glucose levels, the simplified metabolic circuitry maintained glucose levels and showed fatty acid homeostasis. When the system was confronted with elevated levels of glucose, however, its response was immensely altered, and in the presence of insulin, the hyperglycemic metabolite dynamics could be modulated [319]. The system was further combined with microfluidics and adopted to model central obesity featuring systemic and vascular inflammation [273] (Fig. 5B). The relative scaling of the interconnected hepatic, adipose and endothelial compartments was varied to adapt the level of adiposity (healthy: 12% WAT; overweight: 25% WAT; obese: 35% WAT). When exposed to adiposity, the system reacted in an synergic pro-inflammatory response and vascular stress as reflected by expression of E-selectin and von Willebrand factor (both markers for endothelial stress) and expression of IL-6 and monocyte chemoattractant protein-1 (MCP-1) (both markers for systemic stress) [273]. However, it is noteworthy that for the liver compartment hepatocytes of the HepG2 cell line were used; as discussed in section 3.2.3, this hepatoma cell line is not well suited for studying human metabolism. Thus, reconsidering the hepatic cell source of this model is mandatory to further enhance the model's relevance in terms of metabolic research. Still, these two studies form a basis for a microfluidic obesity model with great potential for testing of anti-inflammatory and anti-obesity medications.

3.2.7. Models of diabetic complications

The impact of DM on the human body is not restricted to the respective organs associated to either T1 or T2DM, but the constantly elevated blood glucose levels also cause severe chronic or acute co-morbidities by provoking micro- or macrovascular damages or neuronal injuries. In the following sections, some in vitro studies focusing on models of diabetic complications will be reviewed.

3.2.7.1. Diabetic nephropathy. Diabetic nephropathy (DN) is the leading cause of end-stage kidney diseases eventually requiring a kidney replacement therapy [320]. With approximately 30–40% of all diabetic patients being affected [321], the incidence of DN is genuinely high. Since many renal cell types are insulin-sensitive [320], and are consequently affected by the systemic insulin resistance, too high blood glucose levels can cause a severe dysfunction of the renal glomerular filtration barrier (i.e. the construct of endothelial cells, basement membrane and podocytes) [321]. The key mechanisms behind the dysfunction of glomeruli are (i) a glomerular insulin resistance, which is (ii) leading to a hampered communication between podocytes and glomerular endothelium, in turn (iii) leading to an inhibited reabsorption of glucose via sodium coupled transporters [320].

Owing to the high prevalence of DN, there is a number of in vitro models targeting elucidation of DN mechanisms and treatment options. Besides multiple generic kidney-on-a-chip systems [322–325], Wang and Tao et al. developed a glomerulus-on-a-chip specifically designed as a disease model for DN [321]. The chip emulates the glomerular microenvironment including the glomerular filtration barrier. When exposed to hyperglycemic conditions, the glomerular dysfunction and

the barrier's permeability to albumin significantly increased leading to proteinuria [321]. However, the model relies solely on renal tissue from rodents and it remains to be demonstrated whether those findings are consistent with outcomes from a human glomerulus-on-a-chip.

3.2.7.2. Diabetic retinopathy. Diabetic retinopathy (DR) also ranks among the most frequent DM-associated co-morbidities and is a worldwide leading cause for vision loss [326]. It is a progressive disease featuring an early non-proliferative phase followed by a late proliferative stage characterized by pathologic angiogenesis. DR eventually leads to retinal detachment and vision impairment if it remains untreated. The main initiator of DR is hyperglycemia; however, the disease progresses even after blood glucose levels have been re-adjusted to normal levels indicating that DR exhibits a 'metabolic memory' [327]. The effects of hyperglycemia on the retina have been shown in a murine in vitro model of organotypic retinal explant cultures which were exposed to diabetic conditions; under hyperglycemia, the cell death rates of inner retinal neurons and photoreceptors, especially cones, were dramatically increased [326].

In the first phase of DR, the blood-retinal barrier is harmed by the elevated blood glucose levels leading to a permanently increased permeability which, in turn, provokes tissues inflammation. Upon advancing impairment of the blood-retinal barrier, pathologic angiogenesis is initiated in response to the hypoxia, resulting from a decreased transport of oxygen to the retina, presenting a hallmark of DR's proliferative stage [328]. Thus, since pathological endothelial permeability and neovascularization are integral parts of DR disease progression, in vitro models of microvasculature recapitulating angiogenesis or vascular permeability play an important role in DR research. In recent years, a couple of microvessel- or capillary network-on-a-chip systems have been introduced, which focus on angiogenesis and might be helpful in testing anti-angiogenic agents [329–331]. One of those systems also modeled vascular permeability under inflammatory conditions: after having demonstrated an effective barrier functionality, Pauty et al. induced a loss of this function by administering the inflammatory factor thrombin to the in-chip microvasculature [329].

3.2.7.3. Diabetic cardiomyopathy. In general, the incidence rates of cardiovascular diseases are higher in patients affected by diabetes as compared to non-diabetics [332]. This is especially true for hypertensive and ischemic heart diseases but also for diabetic cardiomyopathy, a condition which is not associated to hypertension or coronary artery disease, unlike hypertensive and ischemic diseases [332]. Diabetic cardiomyopathy induces pathological remodeling of the heart muscle and results in impaired heart function [333]. Its hallmarks are initially an impaired diastolic relaxation time and a ventricular hypertrophy followed by a reduced contractility in the second stage which is eventually leading to systolic dysfunction. These defects can be attributed to a decreased insulin sensitivity of the cardiac muscle as well as to a consequential metabolic overload [332]. Since no specific therapy options for diabetic cardiomyopathy exist to date, a number of in vitro models have been developed to mimic diabetic cardiomyopathy phenotypes [153,333–335]. By integrating, for instance, patient-specific hiPSC-derived cardiomyocytes into a platform enabling phenotypic screening various therapeutic strategies were tested for different patient groups; importantly, diabetic patient-derived hiPSCs showed a baseline cardiomyopathy which could be improved by pharmacological intervention [333]. A further diabetic cardiomyopathy model was established by seeding rat ventricular myocytes into collagen scaffolds and culturing them under four different conditions (normal glucose ± insulin; high glucose ± insulin). Cardiac tissues which were exposed to high glucose levels but were not supplied with insulin showed diabetes-induced gene expression, dysfunctional contractility as well as weakened electrical excitability. In comparison, the presence of insulin promoted viability, excitability and healthy gene expression [153]. Testing anti-diabetic drugs with those systems showed the capability to enhance excitability but did not change the cells' gene expression [334,335]. More comprehensive studies on the mechanisms of diabetic cardiomyopathy in vitro could also make use of the variety of complex stem cell-based heart-on-a-chip models introduced in the last years [336–340].

3.2.7.4. Diabetic osteopathy. T2DM-patients also suffer from an increased risk of bone fractures as well as a deferred healing of fractures. This is caused by circulating factors in T2DM patients' blood, which are a consequence of glucose and insulin levels being higher than physiological thresholds. One of those factors is transforming growth factor beta (TGF-β) which was shown to inhibit osteoblast maturation. To better understand the pathobiology behind those blood circulating factors and to ensure an improved identification of other osteoblast maturation inhibitors, Ehnert et al.developed an in vitro model elucidating the impact of circulating factors from T2DM patients' blood sera on bone function. Comparing the differentiation and maturation of human immortalized bone marrow mesenchymal stem cells (SCP-1 cells) in the presence of sera from T2DM patients or control sera (i.e. sera from non-obese as well as obese non-diabetic individuals) revealed significant differences in osteoblast function [341]. These findings indicate that blocking of the circulating factors might present a treatment option for diabetic osteopathy.

4. Conclusion & future perspectives

Due to the burden on global health of DM, there is an enormous need for the development of new approaches for prevention and treatment. DM-associated research hence can have a huge societal as well as economic impact. Even though the key symptom of DM – hyperglycemia – prevails in all types of DM, the causative mechanisms are remarkably different: in T1DM, an immune-mediated depletion of insulin-secreting β-cells leads to the pathologically elevated blood glucose levels; in T2DM, a variety of systemic deficiencies contribute to a combination of i) insulin resistance in glucose-consuming tissues and ii) defective insulin production. The resulting chronic hyperglycemia, in turn, is the cause of many severe comorbidities including long-term damage, dysfunction, and failure of different organs. Still, there is no cure or effective "remission" of DM yet; DM patients are always at risk of relapse even if normoglycemia can be restored by an optimal disease management [342]. The most prominent therapeutic approaches for T1DM include injection of insulin and pancreas or islet cell transplantation, while for T2DM lifestyle modifications and administration of metformin usually are the first choices [65,73]. Despite the abundancy of therapeutic remission options, DM patients' quality of life is still physically and psychologically challenged by their day-to-day disease management demand and their confrontation with the risk of developing diabetes comorbidities [343]. Further, DM therapy is complicated by adverse effects of antidiabetic drugs (hypoglycemia being the most dreaded) [73,124,344] as well as by the multitude of medications that need to be combined to treat the multiple facets of DM [73]. Hence, it is of upmost importance to deepen our knowledge of the mechanisms behind DM and to find new, more effective pharmacological remedies facilitating disease management or, eventually, even an effective cure reversing functionality loss of β-cells or insulin resistance.

Human cohort studies have already contributed valuably to unraveling the genetic foundations linking a certain genotype to a DM-phenotype as well as to correlating distinct lifestyles to different manifestations of DM. To study DM in greater mechanistic detail from a molecular to an organ level, a multitude of diabetic animal models, particularly rodent models, have been developed. Even though they provided many significant insights into DM, translation from animals to humans – as in many other research areas – has proven to be difficult; glucose regulation mechanisms, for instance, were shown to be extremely species-specific [152].

Meeting the needs of developing diabetes models combining both human genetic background and increased physiological relevance,

Table 1
Selection of in vitro diabetes models and cell sources.

	Reference	Model	Pros	Cons
In vitro diabetes models on a stem cell level				
	Iovino et al. [167] Burkart et al. [166]	iPSCs with genetic mutation in insulin receptor (IR-Mut)	• Investigation of defects in an early developmental stage • Independence of differentiation processes	• In vitro environment does not resemble in vivo environment • No cell type specific and systemic effects recapitulated
In vitro immune system models				
	Lin et al. [346] Halilovic et al. [347]	Chemical gradients-on-a-chip for T-cell and neutrophil migration	• Human primary cells • Flexible and precisely controllable chemical gradients	• Very specific chip systems for individual applications covering only a very small aspect of the immune system
	Kim et al. [238]	Leukocyte binding chip integrating cell adhesion molecules (CAM)	• Enables quantification of binding • Flexible choice of CAMs • Recapitulation of inhibitory effect of immunosuppressants	• Artificial surfaces • No cell-cell interactions • Use of cell lines (Kim et al., Zhang et al., Srigunapalan et al., Han et al.) and/or murine cells (Sundd et al., Zhang et al., Srigunapalan et al.)
	Sundd et al. [239]	Rolling of neutrophils on-chip	• Precise control over shear stresses • Non-invasive High-Res imaging	
	Han et al. [348] Zhang et al. [240] Srigunapalan et al. [241]	Transendothelial migration of neutrophils and monocytes on-chip	• Recapitulation of interaction with endothelial cells • In situ monitoring of adhesion and migration of immune cells	
Pancreas (Endocrine)				
Cell Sources		Primary human and animal pancreatic islets	• Gold standard • Physiological functionality	• Non-proliferative • Limited availability • Very expensive • High variability
		Human and rodent-derived insulinoma/β-cell cell lines	• Good availability • Low cost • Standardized	• Mostly rodent lines • Human lines feature insufficient insulin expression and secretion
		Stem cell derived β-like cells	• Good availability • Retain patient-specific information	• Immature state • Inefficient differentiation protocols
Conventional in vitro models	Bhonde et al. [202]	Human primary Langerhans islet culture	• Preservation of the islet structure • Contain all cell types	• Low availability • Requires ethical approval
	Skelin et al. [179] Ravassard et al. [181] Scharfman et al. [182] Benazra et al. [183] Akerman et al. [218]	2D culture of endocrine cells	• Mostly good availability • Simple and cheap • Standardized	• Non-physiological structure and microenvironment
	Hilderink et al. [205] Zuellig et al. [206] Ichihara et al. [207] Lecomte et al. [208] Ramachandran et al. [209] Gao et al. [210] Weber et al. [211] O'sullivan et al. [212] Mendelsohn et al. [213] Karp et al. [214] Hraha et al. (2014) [215] Shinohara et al. (2014) [216]	Pseudo-islets	• Closer to primary islets as monolayer • Increased glucose response	• Time-consuming • No intrinsic structure • No vascularization

(continued on next page)

Table 1 (*continued*)

	Reference	Model	Pros	Cons
Pancreas-on-a-chip	Mohammed et al. [230] Fig. 4A Li et al. [231] Brooks et al. [232]	Microfluidically perfused "macro"-wells	• Precisely controllable perfusion • Flexible & automated system	• "Large" media reservoirs • Non-physiological microenvironment • Primary mouse islets
	Xing et al. [233]	Pumpless islet analysis	• Pumpless perfusion • Single islets • Mouse and Human islets	• Non-physiological microenvironment
	Schulze et al. [234]	Glass-made microfluidic "micro"-wells	• Single pseudo-islets • Small media volumes • Integrated sensing • Controllable oxygen levels	• Complex microfabrication • Non-physiological microenvironment • Rodent cell line pseudo-islets
	Lee et al. [235]	Microfluidic well platform for pseudo-islet formation and analysis	• On-chip formation of pseudo-islets • Small media volumes • Islets shielded from shear forces	• Human cell line
Liver				
Cell Sources		2D culture of primary human hepatocytes (PHHs)	• Close resemblance to in vivo functionality	• Rapid loss of function ex vivo • Limited availability
		Liver cell lines (HepG2 and HepaRG)	• Good availability • Low cost	• Derived from tumor tissue or obtained by genetic engineering • Functional losses compared to PHHs (especially in terms of drug metabolism)
		hiPSC-derived hepatocyte-like cells (hiPSC-Heps)	• Good availability • Retain patient-specific information	• Immature, even fetal state of hepatocytes • Functional losses compared to PHHs (especially in terms of drug metabolism)
Conventional in vitro models	Vickers et al. [254] Starokozhko et al. [255]	Precision-cut liver slices	• Preservation of liver structure • Contain all cell types • Cryopreservation possible	• Rapid loss of functionality • Limited availability • Low throughput character
	Davidson et al. [256] Davidson et al. [247] Lin and Khetani [257] Berger et al. [258] Ware and Khetani [259]	Micropatterned co-cultures (MPCCs) for PHHs/hiPSC-Heps	• Improved functionality of PHHs/Enhanced maturity and longevity of hiPSC-Heps • Responsiveness to feeding and fasting states • Model for the development of hepatic insulin resistance	• No recapitulation of in vivo liver architecture • Limited availability of PHHs/hiPSC-Heps remain immature
Liver-on-a-chip	Esch et al. [265]	Multi-cellular 3D human primary liver cell culture	• Primary human hepatocytes and non-parenchymal cells (primary human fibroblasts, stellate cells and Kupffer cells) • Increased metabolic activity by fluidic cell culture	• Limited recapitulation of physiological structure
	Rennert & Steinborn et al. [264] Fig. 4C Gröger et al. [244]	Microfluidic co-cultures in MOTiF-chips	• Bile and vascular compartment • Co-culture of hepatocytes, stellate cells, endothelial cells as well as tissue macrophages • Integrated sensors • Inflammation-associated dysfunction	• Cell lines
	Schepers et al. [270]	Perfusable 3D spheroid culture system	• Human iPSC-derived hepatocytes • 3D co-culture spheroids integrating fibroblasts	• Limited recapitulation of physiological structure
	Banaeiyan et al. [266]	Very large-scale liver-lobule (VLSLL)-on-a-chip device	• Human iPSC-derived hepatocytes • Stable albumin secretion and urea synthesis	• Large cell numbers required per system
White adipose tissue				
Cell Sources		Primary mature human white adipocytes	• Good accessibility • Retain patient-specific information	• Culture complicated by buoyancy and fragility • No expansion or cryopreservation possible
		Adipogenic progenitor cells (e.g. pre-adipocytes or multipotent stem cell lines)	• Good accessibility • Low cost • Less fragile and mostly non-buoyant • Expandable and cryopreservable	• Mostly multilocular instead of unilocular • Altered secretion of adipose-associated hormones
		hiPSC-derived white adipocytes (hiPSC-WAT)	• Good availability • Retains patient-specific information	• Lack of efficient differentiation protocols • Immature state of adipocytes

			• Less fragile and non-buoyant • Expandable and cryopreservable	
Conventional in vitro models	Barbagallo et al. [282] Abbott et al. [286–289]	Adipogenic differentiation potential of diabetic MSCs 3D silk protein scaffolds for ex vivo white adipose tissue	• Reflection of diabetic MSCs' capacity to differentiate into functional white adipocytes • Successful in modeling adipogenesis of human preadipocytes • 3D model	• Very specific model, limited applicability as in vitro model • No physiological environment • Artificial environment
	Turner et al. [290]	Inflammated Spheroid preadipocyte cultures	• Reduced metabolic function of adipose tissue exposed to pro-inflammatory stimuli	• Murine cell source
	Zagotta et al. [291]	Human adipose in vitro inflammation model	• Human cells • Recapitulation of reduction in production of inflammation--associated cytokines by anti-obesity compound	• Non-physiological 2D model • No mature adipocytes • Monocyte cell line
Adipose-tissue-on-a-chip	Viravaidya and Shuler [292]	Microscale cell culture analog (μCCA)	• Successful study of bioaccumulation of toxins	• Murine cell source • No disease modeling
	Loskill et al. [293] Fig. 4D	WAT-on-a-chip platform to generate physiologically functional WAT	• Chip design adapted to adipose tissue in vivo architecture • Mature adipose tissue featuring physiologically functional lipid metabolism • Long-term functionality over multiple weeks	
	Moraes et al. [294]	Model of adipose glucose metabolism	• Study insulin-triggered glucose uptake in 3T3-L1 adipocytes • Model shows significance of scaling in diabetes in vitro models	
Skeletal muscle				
Cell Sources		Primary satellite cells	• Myogenic differentiation resembles in vivo situation best	• Limited availability
		Immortal cell lines [e.g. C2C12 (mouse), L6 (rat)]	• Good availability • Low cost	• Loss in myogenic differentiation potential compared to primary satellite cells • Rodent cells source
		Myocyte progenitor cells (MSCs, ESCs, myoblasts)	• Good availability • Low cost	• Loss in myogenic differentiation potential compared to primary satellite cells
		hiPSC-derived myofibers	• Good availability • Retain patient-specific information	• State of maturation unclear • Contractile function remains to be validated
Conventional in vitro models	Iovino et al. [307]	hiPSC-myotube model of skeletal muscle insulin resistance (IR-Mut vs. healthy)	• IR-Mut hiPSC-derived myotubes featured deficient insulin signaling and glucose uptake	
	Qazi et al. [297]	Overview over a variety of skeletal muscle in vitro models	• Broad research ongoing in skeletal muscle engineering which is closely linked to biomaterials research	• Focus on large scale skeletal muscle constructs for reconstructive surgery • Recapitulation of in vivo organization and function is still limited • Inadequate states of physiological maturity
Skeletal muscle-on-a-chip	Madden et al. [312] Fig. 4B	Functional microfluidic model of human skeletal muscle	• Chemically & electrically responsive muscle tissue • Non-invasive monitoring of muscle function • Reaction to myopathy-inducing drugs	• No diabetes- or insulin resistance-focus
	Agrawal et al. [313]	Injury model of skeletal muscle	• Dose-dependent response to cardiotoxin	• Murine cell source • No diabetes- or insulin resistance-focus
Multi-organ approaches				
	Nguyen et al. [316]	Endocrine system on chip	• Intestinal-pancreatic co-culture • Analysis of effect of glucose-levels on dynamic profiles of diabetes-associated hormones	• Rodent cell lines • Non-physiological tissue structures • Linear, one-time pass, perfusion
	Bauer et al. [318]	Pancreatic islets and liver spheroids on-a-chip	• Hepatic-pancreatic co-culture • Human pancreatic islets • Media circulation • In vivo-like homeostatic feedback loop	• Hepatic cell line • Relatively large media volumes • Non-physiological tissue structures and local perfusion

human in vitro models of DM are imperatively required. In recent years, hiPSC and OoC technologies have yielded a broad variety of promising in vitro single- and even multi-organ concepts mimicking certain aspects of DM (cf. Table 1). The most relevant cell type for diabetes models is most definitely pancreatic cells. Since only a limited number of human β-cell lines is available, attention has shifted towards differentiating hiPSCs into β-cells. However, a complete maturation of hiPSC-derived β-cells has merely been shown in vivo so far [194,195]. In vitro differentiation of hiPSC-derived β-cells, in contrast, still falls short on achieving physiologically relevant insulin secretion profiles. Likewise, maturity of hiPSCs-derived cells still states a challenge to be tackled for other diabetes-relevant cell types such as hepatocytes [250,251], adipocytes [277] or skeletal muscle cells [299].

With respect to diabetes-relevant OoCs, considerable progress in the development of single- and multi-organ platforms has been achieved but a wide range of challenges and limitations still need to be tackled. The focus of existing pancreas-on-a-chip platforms tends towards evaluation of islets prior to transplantation therapy and not towards a recapitulation of the in vivo physiology on-chip. Owing to their relevance for drug development and toxicity assessment, liver-on-a-chip systems are abundant and versatile while both adipose- as well as skeletal muscle OoCs are rather at earlier stages of development. Owing to the systemic character of DM, especially multi-organ chips are extremely interesting for pathophysiological and pharmacological studies. Yet, the development of platforms featuring numerous organ compartments designed for recapitulating diabetic disease mechanisms is still in its infancy and still lacks the complexity of DM's heterogeneity. Even though the already established single- and multi-organ systems are not yet capable of fully modeling diabetes in vitro and to assess disease mechanisms and associated treatment modalities, they still foreshadow a great potential in terms of diabetic research; some of the discussed platforms, if not explicitly modeling diabetes, even validated functionality by testing the response to antidiabetic drugs.

As future perspective of stem cell-based diabetes in vitro models, the development of a T1DM-on-a-chip and a T2DM-on-a-chip, exploiting hiPSC technology to retrieve all the relevant types of tissue, would fundamentally change diabetic research. Apart from modeling diabetes disease mechanisms and providing drug candidates' safety and efficacy profiles, hiPSC-based diabetes-chips are envisioned to critically impact personalized medicine: Integration of tissues from individual diabetes patients might allow the investigation of personalized treatment options on the level of this respective individual but also on the level of different populations. Given the variety of symptoms and co-morbidities, as well as the individuality of history and course of disease, a patient-centered approach of diabetes management is crucial. The initial adjustment of antidiabetic medication at the time of first diagnosis can be hard and demanding for the patient; moreover, different subpopulations have been shown to respond differently to blood glucose regulating agents [345]. Hence, a personalized diabetes-on-a-chip platform can help retrieving individual treatment recommendations and consequently unburdening diabetes patients.

In conclusion, to date there are no models which can completely recapitulate DM because of the complexity either presented by the impact of the immune system (T1DM) or by its multifactorial character (T2DM). Yet, there are outstanding advancements in stem cell-based in vitro diabetes research that helped gaining a deeper understanding of the disease and might have the potential to find more effective therapy options or maybe even a cure for diabetes in the future.

Acknowledgements

The authors thank Shannon Lee Layland (University Tübingen) for his helpful comments. The authors would like to thank the Fraunhofer-Gesellschaft internal programs Talenta Start (to J.R.) and Attract (601543 to P.L.), and the European Union's Horizon 2020 research and innovation programme under the grant agreement number 645991-2 (to K.S.-L.) and 766884 (to P.L.).

References

[1] I.D. Federation, IDF Diabetes Atlas, 7th edn. International Diabetes Federation, Brussels, Belgium, 2015.
[2] M.Z. Shrayyef, J.E. Gerich, Normal glucose homeostasis, in: L. Poretsky (Ed.), Principles of Diabetes Mellitus, Springer, Boston, MA 2010, pp. 19–35.
[3] American Diabetes Association, Diagnosis and classification of diabetes mellitus, Diabetes Care 37 (2014) S81–S90.
[4] V.M. Schwitzgebel, Many faces of monogenic diabetes, J. Diabetes Invest. 5 (2014) 121–133.
[5] J. Flannick, S. Johansson, P.R. Njolstad, Common and rare forms of diabetes mellitus: towards a continuum of diabetes subtypes, Nat. Rev. Endocrinol. 12 (2016) 394–406.
[6] American Diabetes Association, American Diabetes Association, Classification and diagnosis of diabetes, Diabetes Care 38 (2015) S8–S16.
[7] A. Ben-Haroush, Y. Yogev, M. Hod, Epidemiology of gestational diabetes mellitus and its association with Type 2 diabetes, Diabet. Med. 21 (2004) 103–113.
[8] D. Aune, A. Sen, T. Henriksen, O.D. Saugstad, S. Tonstad, Physical activity and the risk of gestational diabetes mellitus: a systematic review and dose-response meta-analysis of epidemiological studies, Eur. J. Epidemiol. 31 (2016) 967–997.
[9] L. Hartling, D.M. Dryden, A. Guthrie, M. Muise, B. Vandermeer, L. Donovan, Diagnostic thresholds for gestational diabetes and their impact on pregnancy outcomes: a systematic review, Diabet. Med. 31 (2014) 319–331.
[10] M.A. Atkinson, The pathogenesis and natural history of type 1 diabetes, Cold Spring Harb. Perspect. Med. 2 (2012) a007641.
[11] C.L. Vandewalle, M.I. Coeckelberghs, I.H. De Leeuw, M.V. Du Caju, F.C. Schuit, D.G. Pipeleers, F.K. Gorus, Epidemiology, clinical aspects, and biology of IDDM patients under age 40 years: comparison of data from Antwerp with complete ascertainment with data from Belgium with 40% ascertainment. The belgian diabetes registry, Diabetes Care 20 (1997) 1556–1561.
[12] C.C. Patterson, D.J. Carson, D.R. Hadden, N.R. Waugh, S.K. Cole, A case-control investigation of perinatal risk factors for childhood IDDM in Northern Ireland and Scotland, Diabetes Care 17 (1994) 376–381.
[13] J. Boldison, F.S. Wong, Immune and pancreatic β cell interactions in type 1 diabetes, Trends Endocrinol. Metab. 27 (2016) 856–867.
[14] A.A. Chentoufi, M. Palumbo, C. Polychronakos, Proinsulin expression by Hassall's corpuscles in the mouse thymus, Diabetes 53 (2004) 354–359.
[15] J. Derbinski, A. Schulte, B. Kyewski, L. Klein, Promiscuous gene expression in medullary thymic epithelial cells mirrors the peripheral self, Nat. Immunol. 2 (2001) 1032.
[16] M. Pietropaolo, R. Towns, G.S. Eisenbarth, Humoral autoimmunity in type 1 diabetes: prediction, significance, and detection of distinct disease subtypes, Cold Spring Harb. Perspect. Med. 2 (2012) a012831.
[17] X. Wan, E.R. Unanue, Unique features in the presentation of insulin epitopes in autoimmune diabetes: an update, Curr. Opin. Immunol. 46 (2017) 30–37.
[18] T.K. Starr, S.C. Jameson, K.A. Hogquist, Positive and negative selection of T cells, Annu. Rev. Immunol. 21 (2003) 139–176.
[19] S. Nejentsev, J.M. Howson, N.M. Walker, J. Szeszko, S.F. Field, H.E. Stevens, P. Reynolds, M. Hardy, E. King, J. Masters, Localization of type 1 diabetes susceptibility to the MHC class I genes HLA-B and HLA-A, Nature 450 (2007) 887.
[20] P.A. Morel, J.S. Dorman, J.A. Todd, H.O. McDevitt, M. Trucco, Aspartic acid at position 57 of the HLA-DQ beta chain protects against type I diabetes: a family study, Proc. Natl. Acad. Sci. 85 (1988) 8111–8115.
[21] B.O. Roep, T.I. Tree, Immune modulation in humans: implications for type 1 diabetes mellitus, Nat. Rev. Endocrinol. 10 (2014) 229–242.
[22] B. Calderon, J.A. Carrero, M.J. Miller, E.R. Unanue, Cellular and molecular events in the localization of diabetogenic T cells to islets of Langerhans, Proc. Natl. Acad. Sci. 108 (2011) 1561–1566.
[23] A. Willcox, S. Richardson, A. Bone, A. Foulis, N. Morgan, Analysis of islet inflammation in human type 1 diabetes, Clin. Exp. Immunol. 155 (2009) 173–181.
[24] L.E. Padgett, K.A. Broniowska, P.A. Hansen, J.A. Corbett, H.M. Tse, The role of reactive oxygen species and proinflammatory cytokines in type 1 diabetes pathogenesis, Ann. N. Y. Acad. Sci. 1281 (2013) 16–35.
[25] F. Engin, ER stress and development of type 1 diabetes, J. Investig. Med. 64 (2016) 2–6.
[26] F. Engin, A. Yermalovich, T. Nguyen, S. Hummasti, W. Fu, D.L. Eizirik, D. Mathis, G.S. Hotamisligil, Restoration of the unfolded protein response in pancreatic β cells protects mice against type 1 diabetes, Sci. Transl. Med. 5 (2013) 211ra156–211ra156.
[27] C. Polychronakos, Q. Li, Understanding type 1 diabetes through genetics: advances and prospects, Nat. Rev. Genet. 12 (2011) 781.
[28] J.A. Noble, Immunogenetics of type 1 diabetes: a comprehensive review, J. Autoimmun. 64 (2015) 101–112.
[29] J. Neefjes, M.L. Jongsma, P. Paul, O. Bakke, Towards a systems understanding of MHC class I and MHC class II antigen presentation, Nat. Rev. Immunol. 11 (2011) 823.
[30] A.L. Dixon, L. Liang, M.F. Moffatt, W. Chen, S. Heath, K.C. Wong, J. Taylor, E. Burnett, I. Gut, M. Farrall, A genome-wide association study of global gene expression, Nat. Genet. 39 (2007) 1202.
[31] A.L. Corper, T. Stratmann, V. Apostolopoulos, C.A. Scott, K.C. Garcia, A.S. Kang, I.A. Wilson, L. Teyton, A structural framework for deciphering the link between I-Ag7 and autoimmune diabetes, Science 288 (2000) 505–511.

[32] J.A. Noble, A.M. Valdes, M.D. Varney, J.A. Carlson, P. Moonsamy, A.L. Fear, J.A. Lane, E. Lavant, R. Rappner, A. Louey, HLA class I and genetic susceptibility to type 1 diabetes, Diabetes 59 (2010) 2972–2979.
[33] P. Rotwein, S. Yokoyama, D. Didier, J. Chirgwin, Genetic analysis of the hypervariable region flanking the human insulin gene, Am. J. Hum. Genet. 39 (1986) 291.
[34] B.J. Barratt, F. Payne, C.E. Lowe, R. Hermann, B.C. Healy, D. Harold, P. Concannon, N. Gharani, M.I. McCarthy, M.G. Olavesen, Remapping the insulin gene/IDDM2 locus in type 1 diabetes, Diabetes 53 (2004) 1884–1889.
[35] C.Q. Cai, T. Zhang, M.B. Breslin, M. Giraud, M.S. Lan, Both polymorphic variable number of tandem repeats and autoimmune regulator modulate differential expression of insulin in human thymic epithelial cells, Diabetes 60 (2011) 336–344.
[36] B. Kyewski, L. Klein, A central role for central tolerance, Annu. Rev. Immunol. 24 (2006) 571–606.
[37] D. Smyth, J.D. Cooper, J.E. Collins, J.M. Heward, J.A. Franklyn, J.M. Howson, A. Vella, S. Nutland, H.E. Rance, L. Maier, Replication of an association between the lymphoid tyrosine phosphatase locus (LYP/PTPN22) with type 1 diabetes, and evidence for its role as a general autoimmunity locus, Diabetes 53 (2004) 3020–3023.
[38] M. Heinig, E. Petretto, C. Wallace, L. Bottolo, M. Rotival, H. Lu, Y. Li, R. Sarwar, S.R. Langley, A. Bauerfeind, A trans-acting locus regulates an anti-viral expression network and type 1 diabetes risk, Nature 467 (2010) 460.
[39] H. Kato, O. Takeuchi, S. Sato, M. Yoneyama, M. Yamamoto, K. Matsui, S. Uematsu, A. Jung, T. Kawai, K.J. Ishii, Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses, Nature 441 (2006) 101.
[40] R. Tattersall, D. Pyke, Diabetes in identical twins, Lancet 300 (1972) 1120–1125.
[41] R. Zinkernagel, H. Hengartner, L. Stitz, On the role of viruses in the evolution of immune responses, Br. Med. Bull. 41 (1985) 92–97.
[42] T. Rodriguez-Calvo, S. Sabouri, F. Anquetil, M.G. von Herrath, The viral paradigm in type 1 diabetes: Who are the main suspects? Autoimmun. Rev. 15 (2016) 964–969.
[43] N.G. Morgan, S.J. Richardson, Enteroviruses as causative agents in type 1 diabetes: loose ends or lost cause? Trends Endocrinol. Metab. 25 (2014) 611–619.
[44] O. Cinek, L.C. Stene, L. Kramna, G. Tapia, S. Oikarinen, E. Witsø, T. Rasmussen, P.A. Torjesen, H. Hyöty, K.S. Rønningen, Enterovirus RNA in longitudinal blood samples and risk of islet autoimmunity in children with a high genetic risk of type 1 diabetes: the MIDIA study, Diabetologia 57 (2014) 2193–2200.
[45] G. Tapia, O. Cinek, T. Rasmussen, E. Witsø, B. Grinde, L.C. Stene, K.S. Rønningen, Human enterovirus RNA in monthly fecal samples and islet autoimmunity in Norwegian children with high genetic risk for type 1 diabetes, Diabetes Care 34 (2011) 151–155.
[46] L.C. Stene, S. Oikarinen, H. Hyöty, K.J. Barriga, J.M. Norris, G. Klingensmith, J.C. Hutton, H.A. Erlich, G.S. Eisenbarth, M. Rewers, Enterovirus infection and progression from islet autoimmunity to type 1 diabetes, Diabetes 59 (2010) 3174–3180.
[47] L. Krogvold, B. Edwin, T. Buanes, J. Ludvigsson, O. Korsgren, H. Hyöty, G. Frisk, K.F. Hanssen, K. Dahl-Jørgensen, Pancreatic biopsy by minimal tail resection in live adult patients at the onset of type 1 diabetes: experiences from the DiViD study, Diabetologia 57 (2014) 841–843.
[48] L. Krogvold, B. Edwin, T. Buanes, G. Frisk, O. Skog, M. Anagandula, O. Korsgren, D. Undlien, M. Eike, S.J. Richardson, Detection of a low-grade enteroviral infection in the islets of langerhans of living patients newly diagnosed with type 1 diabetes, Diabetes 64 (5) (2015) 1682–1687.
[49] G.R. Gallagher, M.A. Brehm, R.W. Finberg, B.A. Barton, L.D. Shultz, D.L. Greiner, R. Bortell, J.P. Wang, Viral infection of engrafted human islets leads to diabetes, Diabetes 64 (2015) 1358–1369.
[50] J.O. Nyalwidhe, G.R. Gallagher, L. Glenn, M.A. Morris, P. Vangala, A. Jurczyk, R. Bortell, D.M. Harlan, J.P. Wang, J.L. Nadler, Coxsackievirus-induced proteomic alterations in primary human islets provide insights for the etiology of diabetes, J. Endocrine Soc. 1 (10) (2017) 1272–1286.
[51] Y. Ding, Y. Xu, X. Shuai, X. Shi, X. Chen, W. Huang, Y. Liu, X. Liang, Z. Zhang, D. Su, Reg3α overexpression protects pancreatic β cells from cytokine-induced damage and improves islet transplant outcome, Mol. Med. 20 (2014) 548.
[52] M. Lundberg, L. Krogvold, E. Kuric, K. Dahl-Jørgensen, O. Skog, Expression of interferon-stimulated genes in insulitic pancreatic islets of patients recently diagnosed with type 1 diabetes, Diabetes 65 (19) (2016) 3104–3110.
[53] V.S. Jean-Baptiste, C.-Q. Xia, M.J. Clare-Salzler, M.S. Horwitz, Type 1 diabetes and type 1 interferonopathies: localization of a type 1 common thread of virus infection in the pancreas, EBioMedicine 22 (2017) 10–17.
[54] N. Qaisar, S. Lin, G. Ryan, C. Yang, S.R. Oikemus, M.H. Brodsky, R. Bortell, J.P. Mordes, J.P. Wang, A critical role for the type i interferon receptor in virus-induced autoimmune diabetes in rats, Diabetes 66 (2017) 145–157.
[55] H. Honkanen, S. Oikarinen, N. Nurminen, O.H. Laitinen, H. Huhtala, J. Lehtonen, T. Ruokoranta, M.M. Hankaniemi, V. Lecouturier, J.W. Almond, Detection of enteroviruses in stools precedes islet autoimmunity by several months: possible evidence for slowly operating mechanisms in virus-induced autoimmunity, Diabetologia 60 (2017) 424–431.
[56] A. Giongo, K.A. Gano, D.B. Crabb, N. Mukherjee, L.L. Novelo, G. Casella, J.C. Drew, J. Ilonen, M. Knip, H. Hyöty, Toward defining the autoimmune microbiome for type 1 diabetes, ISME J. 5 (2011) 82.
[57] M. Knip, H. Siljander, The role of the intestinal microbiota in type 1 diabetes mellitus, Nat. Rev. Endocrinol. 12 (2016) 154–167.
[58] M. Rewers, J. Ludvigsson, Environmental risk factors for type 1 diabetes, Lancet 387 (2016) 2340–2348.
[59] S.T. Jerram, M.N. Dang, R.D. Leslie, The role of epigenetics in type 1 diabetes, Current Diabetes Reports 17 (2017) 89.
[60] J. Chia, J. McRae, S. Kukuljan, K. Woodford, R. Elliott, B. Swinburn, K. Dwyer, A1 beta-casein milk protein and other environmental pre-disposing factors for type 1 diabetes, Nutr. Diabetes 7 (2017), e274.
[61] S. Schoen, S. Jergens, J. Barbaresko, U. Nöthlings, M. Kersting, T. Remer, M. Stelmach-Mardas, A.-G. Ziegler, S. Hummel, Diet quality during infancy and early childhood in children with and without risk of type 1 diabetes: a DEDIPAC study, Nutrients 9 (2017) 48.
[62] F. Prodam, A. Chiocchetti, U. Dianzani, Diet as a strategy for type 1 diabetes prevention, Cell. Mol. Immunol. 15 (1) (2017) 1–4.
[63] D.T. Karamitsos, The story of insulin discovery, Diabetes Res. Clin. Pract. 93 (2011) S2–S8.
[64] R. Aathira, V. Jain, Advances in management of type 1 diabetes mellitus, World J. Diabetes 5 (2014) 689.
[65] A.D. Association, Approaches to glycemic treatment, Diabetes Care 39 (2016) S52–S59.
[66] C. Levetan, L.L. Want, C. Weyer, S.A. Strobel, J. Crean, Y. Wang, D.G. Maggs, O.G. Kolterman, M. Chandran, S.R. Mudaliar, Impact of pramlintide on glucose fluctuations and postprandial glucose, glucagon, and triglyceride excursions among patients with type 1 diabetes intensively treated with insulin pumps, Diabetes Care 26 (2003) 1–8.
[67] S.K. Lyons, J.M. Hermann, K.M. Miller, S.E. Hofer, N.C. Foster, B.M. Rami-Merhar, G. Aleppo, J. Seufert, L.A. DiMeglio, T. Danne, Use of adjuvant pharmacotherapy in type 1 diabetes: international comparison of 49,996 individuals in the prospective diabetes follow-up and T1D exchange registries, Diabetes Care 40 (2017) e139–e140.
[68] P. Martyn-Nemeth, S.S. Farabi, D. Mihailescu, J. Nemeth, L. Quinn, Fear of hypoglycemia in adults with type 1 diabetes: impact of therapeutic advances and strategies for prevention-a review, J. Diabetes Complicat. 30 (2016) 167–177.
[69] A.J. Shapiro, M. Pokrywczynska, C. Ricordi, Clinical pancreatic islet transplantation, Nat. Rev. Endocrinol. 13 (5) (2017) 268–277.
[70] A.C. Farney, D.E. Sutherland, E.C. Opara, Evolution of islet transplantation for the last 30 years, Pancreas 45 (2016) 8–20.
[71] Collaborative Islet Transplant Registry, Eighth Annual Report, CITR www.citregistry.org 2014.
[72] B.J. Hering, W.R. Clarke, N.D. Bridges, T.L. Eggerman, R. Alejandro, M.D. Bellin, K. Chaloner, C.W. Czarniecki, J.S. Goldstein, L.G. Hunsicker, Phase 3 trial of transplantation of human islets in type 1 diabetes complicated by severe hypoglycemia, Diabetes Care 39 (2016) 1230–1240.
[73] R.A. DeFronzo, E. Ferrannini, L. Groop, R.R. Henry, W.H. Herman, J.J. Holst, F.B. Hu, C.R. Kahn, I. Raz, G.I. Shulman, D.C. Simonson, M.A. Testa, R. Weiss, Type 2 diabetes mellitus, Nat Rev Dis Primers 1 (2015) 15019.
[74] S.E. Khan, M.E. Cooper, S. Del Prato, Pathophysiology and treatment of type 2 diabetes: prespectives on the past, present and future, Lancet 383 (2014) 1068–1083.
[75] R.A. Defronzo, From the triumvirate to the ominous octet: A new paradigm for the treatment of type 2 diabetes mellitus, Diabetes 58 (2009) 773–795.
[76] E. Ferrannini, A. Mari, Beta-Cell function in type 2 diabetes, Metabolism 63 (2014) 1217–1227.
[77] G.M. Reaven, Role of insulin resistance in human disease, Diabetes 37 (1988) 1595–1607.
[78] A. Chaudhury, C. Duvoor, V.S.R. Dendi, S. Kraleti, A. Chada, R. Ravilla, A. Marco, N.S. Shekhawat, M.T. Montales, K. Kuriakose, Clinical review of antidiabetic drugs: Implications for type 2 diabetes mellitus management, Front Endocrinol. 8 (2017) 6.
[79] Varman T. Samuel, Gerald I. Shulman, Mechanisms for insulin resistance: common threads and missing links, Cell 148 (2012) 852–871.
[80] V.T. Samuel, G.I. Shulman, The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux, J. Clin. Invest. 126 (2016) 12–22.
[81] R.A. DeFronzo, E. Ferrannini, D.C. Simonson, Fasting hyperglycemia in non-insulin-dependent diabetes mellitus: contributions of excessive hepatic glucose production and impaired tissue glucose uptake, Metabolism 38 (1989) 387–395.
[82] J.G. Jones, Hepatic glucose and lipid metabolism, Diabetologia 59 (2016) 1098–1103.
[83] B.C. Lee, M.S. Kim, M. Pae, Y. Yamamoto, D. Eberle, T. Shimada, N. Kamei, H.S. Park, S. Sasorith, J.R. Woo, J. You, W. Mosher, H.J. Brady, S.E. Shoelson, J. Lee, Adipose natural killer cells regulate adipose tissue macrophages to promote insulin resistance in obesity, Cell Metab. 23 (2016) 685–698.
[84] A. Guilherme, J.V. Virbasius, V. Puri, M.P. Czech, Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes, Nat. Rev. Mol. Cell Biol. 9 (2008) 367–377.
[85] F. Artunc, E. Schleicher, C. Weigert, A. Fritsche, N. Stefan, H.U. Haring, The impact of insulin resistance on the kidney and vasculature, Nat. Rev. Nephrol. 12 (2016) 721–737.
[86] S. Kullmann, M. Heni, M. Hallschmid, A. Fritsche, H. Preissl, H.-U. Häring, Brain insulin resistance at the crossroads of metabolic and cognitive disorders in humans, Physiol. Rev. 96 (2016) 1169–1209.
[87] Daniel A. Winer, H. Luck, S. Tsai, S., Winer, the intestinal immune system in obesity and insulin resistance, Cell Metab. 23 (2016) 413–426.
[88] V. De Tata, Age-related impairment of pancreatic Beta-cell function: pathophysiological and cellular mechanisms, Front. Endocrinol. (2014) 138.
[89] A.P. Morris, B.F. Voight, T.M. Teslovich, T. Ferreira, A.V. Segrè, V. Steinthorsdottir, R.J. Strawbridge, H. Khan, H. Grallert, A. Mahajan, I. Prokopenko, H.M. Kang, C. Dina, T. Esko, R.M. Fraser, S. Kanoni, A. Kumar, V. Lagou, C. Langenberg, J.A. Luan, C.M. Lindgren, M. Müller-Nurasyid, S. Pechlivanis, N.W. Rayner, L.J. Scott, S. Wiltshire, L. Yengo, L. Kinnunen, E.J. Rossin, S. Raychaudhuri, A.D. Johnson, A.S. Dimas, R.J.F. Loos, S. Vedantam, H. Chen, J.C. Florez, C. Fox, C.-T. Liu, D. Rybin, D.J. Couper, W.H.L. Kao, M. Li, M.C. Cornelis, P. Kraft, Q. Sun, R.M. van Dam, H.M. Stringham, P.S. Chines, K. Fischer, P. Fontanillas, O.L. Holmen, S.E. Hunt, A.U. Jackson, A. Kong, R. Lawrence, J. Meyer, J.R.B. Perry, C.G.P. Platou, S. Potter, E. Rehnberg, N. Robertson, S. Sivapalaratnam, A. Stančáková, K. Stirrups, G. Thorleifsson, E. Tikkanen, A.R. Wood, P. Almgren, M. Atalay, R. Benediktsson, L.L.

Bonnycastle, N. Burtt, J. Carey, G. Charpentier, A.T. Crenshaw, A.S.F. Doney, M. Dorkhan, S. Edkins, V. Emilsson, E. Eury, T. Forsen, K. Gertow, B. Gigante, G.B. Grant, C.J. Groves, C. Guiducci, C. Herder, A.B. Hreidarsson, J. Hui, A. James, A. Jonsson, W. Rathmann, N. Klopp, J. Kravic, K. Krjutškov, C. Langford, K. Leander, E. Lindholm, S. Lobbens, S. Männistö, G. Mirza, T.W. Mühleisen, B. Musk, M. Parkin, L. Rallidis, J. Saramies, B. Sennblad, S. Shah, G. Sigurðsson, A. Silveira, G. Steinbach, B. Thorand, J. Trakalo, F. Veglia, R. Wennauer, W. Winckler, D. Zabaneh, H. Campbell, C. van Duijn, A.G. Uitterlinden, A. Hofman, E. Sijbrands, G.R. Abecasis, K.R. Owen, E. Zeggini, M.D. Trip, N.G. Forouhi, A.-C. Syvänen, J.G. Eriksson, L. Peltonen, M.M. Nöthen, B. Balkau, C.N.A. Palmer, V. Lyssenko, T. Tuomi, B. Isomaa, D.J. Hunter, L. Qi, C. Wellcome Trust Case Control, M. Investigators, G. Consortium, A.-T.D. Consortium, S.D. Consortium, A.R. Shuldiner, M. Roden, I. Barroso, T. Wilsgaard, J. Beilby, K. Hovingh, J.F. Price, J.F. Wilson, R. Rauramaa, T.A. Lakka, L. Lind, G. Dedoussis, I. Njølstad, N.L. Pedersen, K.-T. Khaw, N.J. Wareham, S.M. Keinanen-Kiukaanniemi, T.E. Saaristo, E. Korpi-Hyövälti, J. Saltevo, M. Laakso, J. Kuusisto, A. Metspalu, F.S. Collins, K.L. Mohlke, R.N. Bergman, J. Tuomilehto, B.O. Boehm, C. Gieger, K. Hveem, S. Cauchi, P. Froguel, D. Baldassarre, E. Tremoli, S.E. Humphries, D. Saleheen, J. Danesh, E. Ingelsson, S. Ripatti, V. Salomaa, R. Erbel, K.-H. Jöckel, S. Moebus, A. Peters, T. Illig, U. de Faire, A. Hamsten, A.D. Morris, P.J. Donnelly, T.M. Frayling, A.T. Hattersley, E. Boerwinkle, O. Melander, S. Kathiresan, P.M. Nilsson, P. Deloukas, U. Thorsteinsdottir, L.C. Groop, K. Stefansson, F. Hu, J.S. Pankow, J. Dupuis, J.B. Meigs, D. Altshuler, M. Boehnke, M.I. McCarthy, Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes, Nat. Genet. 44 (2012) 981–990.

[90] M. Prentki, C.J. Nolan, Islet β cell failure in type 2 diabetes, J. Clin. Investig. 116 (2006) 1802–1812.

[91] R.A. DeFronzo, Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The claude bernard lecture 2009, Diabetologia 53 (2010) 1270–1287.

[92] J. Dooley, L. Tian, S. Schonefeldt, V. Delghingaro-Augusto, J.E. Garcia-Perez, E. Pasciuto, D. Di Marino, E.J. Carr, N. Oskolkov, V. Lyssenko, D. Franckaert, V. Lagou, L. Overbergh, J. Vandenbussche, J. Allemeersch, G. Chabot-Roy, J.E. Dahlstrom, D.R. Laybutt, N. Petrovsky, L. Socha, K. Gevaert, A.M. Jetten, D. Lambrechts, M.A. Linterman, C.C. Goodnow, C.J. Nolan, S. Lesage, S.M. Schlenner, A. Liston, Genetic predisposition for beta cell fragility underlies type 1 and type 2 diabetes, Nat. Genet. 48 (2016) 519–527.

[93] A. Liston, J.A. Todd, V. Lagou, Beta-cell fragility as a common underlying risk factor in type 1 and type 2 diabetes, Trends Mol. Med. 23 (2017) 181–194.

[94] C.B. Giorda, G.T. Russo, S. Cercone, S. De Cosmo, A. Nicolucci, D. Cucinotta, Incidence and correlated factors of beta cell failure in a 4-year follow-up of patients with type 2 diabetes: a longitudinal analysis of the BETADECLINE study, Acta Diabetol. 53 (2016) 761–767.

[95] J.L. Leahy, I.B. Hirsch, K.A. Peterson, D. Schneider, Targeting β-cell function early in the course of therapy for type 2 diabetes mellitus, J. Clin. Endocrinol. Metab. 95 (2010) 4206–4216.

[96] J. Rahier, Y. Guiot, R.M. Goebbels, C. Sempoux, J.C. Henquin, Pancreatic β-cell mass in European subjects with type 2 diabetes, Diabetes. Obes. Metab. 10 (2008) 32–42.

[97] T. Mezza, G. Muscogiuri, G.P. Sorice, G. Clemente, J. Hu, A. Pontecorvi, J.J. Holst, A. Giaccari, R.N. Kulkarni, Insulin resistance alters islet morphology in nondiabetic humans, Diabetes 63 (2014) 994–1007.

[98] A.E. Butler, J. Janson, S. Bonner-Weir, R. Ritzel, R.A. Rizza, P.C. Butler, β-cell deficit and increased β-cell apoptosis in humans with type 2 diabetes, Diabetes 52 (2003) 102–110.

[99] S. Deng, M. Vatamaniuk, X. Huang, N. Doliba, M.-M. Lian, A. Frank, E. Velidedeoglu, N.M. Desai, B. Koeberlein, B. Wolf, C.F. Barker, A. Naji, F.M. Matschinsky, J.F. Markmann, Structural and functional abnormalities in the islets isolated from type 2 diabetic subjects, Diabetes 53 (2004) 624–632.

[100] M. Masini, M. Bugliani, R. Lupi, S. del Guerra, U. Boggi, F. Filipponi, L. Marselli, P. Masiello, P. Marchetti, Autophagy in human type 2 diabetes pancreatic beta cells, Diabetologia 52 (2009) 1083–1086.

[101] E. Ferrannini, A. Natali, E. Muscelli, P.M. Nilsson, A. Golay, M. Laakso, H. Beck-Nielsen, A. Mari, Natural history and physiological determinants of changes in glucose tolerance in a non-diabetic population: the RISC study, Diabetologia 54 (2011) 1507–1516.

[102] Guy A. Rutter, Timothy J. Pullen, David J. Hodson, A. Martinez-Sanchez, Pancreatic β-cell identity, glucose sensing and the control of insulin secretion, Biochem. J. 466 (2015) 203–218.

[103] C. Talchai, S. Xuan, H.V. Lin, L. Sussel, D. Accili, Pancreatic beta cell dedifferentiation as a mechanism of diabetic beta cell failure, Cell 150 (2012) 1223–1234.

[104] M.K.H. Chowdhury, N. Turner, N.L. Bentley, A. Das, L.E. Wu, D. Richani, S. Bustamante, R.B. Gilchrist, M.J. Morris, P.R. Shepherd, G.C. Smith, Niclosamide reduces glucagon sensitivity via hepatic PKA inhibition in obese mice: Implications for glucose metabolism improvements in type 2 diabetes, Sci. Rep. 7 (2017) 40159.

[105] C. Bonner, J. Kerr-Conte, V. Gmyr, G. Queniat, E. Moerman, J. Thevenet, C. Beaucamps, N. Delalleau, I. Popescu, W.J. Malaisse, A. Sener, B. Deprez, A. Abderrahmani, B. Staels, F. Pattou, Inhibition of the glucose transporter SGLT2 with dapagliflozin in pancreatic alpha cells triggers glucagon secretion, Nat. Med. 21 (2015) 512–517.

[106] J.P.H. Wilding, The role of the kidneys in glucose homeostasis in type 2 diabetes: clinical implications and therapeutic significance through sodium glucose co-transporter 2 inhibitors, Metabolism 63 (2014) 1228–1237.

[107] J.J. Holst, F.K. Knop, T. Vilsbøll, T. Krarup, S. Madsbad, Loss of incretin effect is a specific, important, and early characteristic of type 2 diabetes, Diabetes Care 34 (2011) S251–S257.

[108] J.J. Holst, J. Pedersen, N.J. Wewer Albrechtsen, F.K. Knop, The gut: a key to the pathogenesis of type 2 diabetes? Metab. Syndr. Relat. Disord. 15 (2017) 259–262.

[110] H. Toplak, F. Hoppichler, T.C. Wascher, K. Schindler, B. Ludvik, Adipositas und Typ 2 diabetes, Wien. Klin. Wochenschr. 128 (2016) 196–200.

[111] N.T. Ayas, D.P. White, W.K. Al-Delaimy, J.E. Manson, M.J. Stampfer, F.E. Speizer, S. Patel, F.B. Hu, A prospective study of self-reported sleep duration and incident diabetes in women, Diabetes Care 26 (2003) 380–384.

[112] F.P. Cappuccio, L. D'Elia, P. Strazzullo, M.A. Miller, Quantity and quality of sleep and incidence of type 2 diabetes: a systematic review and meta-analysis, Diabetes Care 33 (2010) 414–420.

[113] A. Pan, E.S. Schernhammer, Q. Sun, F.B. Hu, Rotating night shift work and risk of type 2 diabetes: two prospective cohort studies in women, PLoS Med. 8 (2011), e1001141.

[114] P. Tuso, Prediabetes and lifestyle modification: time to prevent a preventable disease, Perm J. 18 (2014) 88–93.

[115] A. Kautzky-Willer, J. Harreiter, G. Pacini, Sex and gender differences in risk, pathophysiology and complications of type 2 diabetes mellitus, Endocr. Rev. 37 (2016) 278–316.

[116] A.D. Association, Standards of medical care in diabetes 2014, Diabetes Care 37 (2014) 386–390.

[117] C. Bennett, M. Guo, S. Dharmage, HbA1c as a screening tool for detection of type 2 diabetes: a systematic review, Diabet. Med. 24 (2007) 333–343.

[118] A.D. Association, Standards of medical care in diabetes—2014, Diabetes Care 37 (2014) S14–S80.

[119] B. Viollet, B. Guigas, N.S. Garcia, J. Leclerc, M. Foretz, F. Andreelli, Cellular and molecular mechanisms of metformin: an overview, Clin. Sci. 122 (2012) 253–270.

[120] K.B. Harris, D.J. McCarty, Efficacy and tolerability of glucagon-like peptide-1 receptor agonists in patients with type 2 diabetes mellitus, Ther. Adv. Endocrinol. Metab. 6 (2015) 3–18.

[121] S. Taylor, K.B. Harris, The clinical efficacy and safety of sodium glucose cotransporter-2 inhibitors in adults with type 2 diabetes mellitus, Pharmacotherapy 33 (9) (2013) 984–999.

[122] M. Monami, I. Dicembrini, D. Martelli, E. Mannucci, Safety of dipeptidyl peptidase-4 inhibitors: a meta-analysis of randomized clinical trials, Curr. Med. Res. Opin. 27 (2011) 57–64.

[123] S.E. Inzucchi, R.M. Bergenstal, J.B. Buse, M. Diamant, E. Ferrannini, M. Nauck, A.L. Peters, A. Tsapas, R. Wender, D.R. Matthews, Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD), Diabetes Care 35 (2012).

[124] S.E. Inzucchi, R.M. Bergenstal, J.B. Buse, M. Diamant, E. Ferrannini, M. Nauck, A.L. Peters, A. Tsapas, R. Wender, D.R. Matthews, Management of hyperglycaemia in type 2 diabetes, 2015: a patient-centred approach update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes, Diabetologia 58 (2015) 429–442.

[125] J.M. Forbes, M.E. Cooper, Mechanisms of diabetic complications, Physiol. Rev. 93 (2013) 137–188.

[126] D.M. Nathan, B. Zinman, P.A. Cleary, J.-Y.C. Backlund, S. Genuth, R. Miller, T.J. Orchard, D. Control, C. Trial, E.o.D. Interventions, C.R. Group, Modern-day clinical course of type 1 diabetes mellitus after 30 years' duration: the diabetes control and complications trial/epidemiology of diabetes interventions and complications and Pittsburgh epidemiology of diabetes complications experience (1983-2005), Arch. Intern. Med. 169 (2009) 1307.

[127] L. Litwak, S.-Y. Goh, Z. Hussein, R. Malek, V. Prusty, M.E. Khamseh, Prevalence of diabetes complications in people with type 2 diabetes mellitus and its association with baseline characteristics in the multinational A1chieve study, Diabetol. Metab. Syndr. 5 (2013) 57.

[128] J.M. Forbes, A.K. Fotheringham, Vascular complications in diabetes: old messages, new thoughts, Diabetologia (2017) 1–10.

[129] J.A. Beckman, M.A. Creager, Vascular complications of diabetes, Circ. Res. 118 (2016) 1771–1785.

[130] C.P. Domingueti, L.M.S.A. Dusse, M. das Graças Carvalho, L.P. de Sousa, K.B. Gomes, A.P. Fernandes, Diabetes mellitus: the linkage between oxidative stress, inflammation, hypercoagulability and vascular complications, J. Diabetes Complicat. 30 (2016) 738–745.

[131] R. Pop-Busui, A.J. Boulton, E.L. Feldman, V. Bril, R. Freeman, R.A. Malik, J.M. Sosenko, D. Ziegler, Diabetic neuropathy: a position statement by the American Diabetes Association, Diabetes Care 40 (2017) 136–154.

[132] Karen E. Porter, K., Riches, the vascular smooth muscle cell: a therapeutic target in type 2 diabetes? Clin. Sci. 125 (2013) 167–182.

[133] G. Jia, M.A. Hill, J.R. Sowers, Diabetic cardiomyopathy: an update of mechanisms contributing to this clinical entity, Circ. Res. 122 (2018) 624–638.

[134] S. Boudina, E.D. Abel, Diabetic cardiomyopathy revisited, Circulation 115 (2007) 3213–3223.

[135] M.B. Kapp, Ethical and legal issues in research involving human subjects: do you want a piece of me? J. Clin. Pathol. 59 (2006) 335–339.

[136] M.I. McCarthy, Genetics of T2DM in 2016: Biological and translational insights from T2DM genetics, Nat. Rev. Endocrinol. 13 (2017) 71–72.

[137] F. Pociot, Type 1 diabetes genome-wide association studies: not to be lost in translation, Clin. Transplat. Immunol. 6 (2017), e162.

[138] L. Field, Genetic linkage and association studies of Type I diabetes: challenges and rewards, Diabetologia 45 (2002) 21–35.

[139] P.M. Visscher, N.R. Wray, Q. Zhang, P. Sklar, M.I. McCarthy, M.A. Brown, J. Yang, 10 years of GWAS discovery: biology, function, and translation, Am. J. Hum. Genet. 101 (2017) 5–22.

[140] S. Hummel, A. Beyerlein, R. Tamura, U. Uusitalo, C.A. Aronsson, J. Yang, A. Riikonen, Å. Lernmark, M.J. Rewers, W.A. Hagopian, First infant formula type and risk of islet autoimmunity in the environmental determinants of diabetes in the young (TEDDY) study, Diabetes Care 40 (2017) 398–404.
[141] J.P. Krischer, X. Liu, Å. Lernmark, W.A. Hagopian, M.J. Rewers, J.-X. She, J. Toppari, A.-G. Ziegler, B. Akolkar, T.S. Group, The influence of type 1 diabetes genetic susceptibility regions, age, sex, and family history to the progression from multiple autoantibodies to type 1 diabetes: a TEDDY study report, Diabetes 66 (12) (2017) 3122–3129.
[142] J.P. Krischer, K.F. Lynch, Å. Lernmark, W.A. Hagopian, M.J. Rewers, J.-X. She, J. Toppari, A.-G. Ziegler, B. Akolkar, T.S. Group, Genetic and environmental interactions modify the risk of diabetes-related autoimmunity by 6 years of age: the teddy study, Diabetes Care 40 (2017) 1194–1202.
[143] A.K. Steck, K. Vehik, E. Bonifacio, A. Lernmark, A.-G. Ziegler, W.A. Hagopian, J. She, O. Simell, B. Akolkar, J. Krischer, Predictors of progression from the appearance of islet autoantibodies to early childhood diabetes: The Environmental Determinants of Diabetes in the Young (TEDDY), Diabetes Care 38 (2015) 808–813.
[144] H.E. Larsson, K. Vehik, M.J. Haller, X. Liu, B. Akolkar, W. Hagopian, J. Krischer, Å. Lernmark, J.-X. She, O. Simell, Growth and risk for islet autoimmunity and progression to type 1 diabetes in early childhood: the environmental determinants of diabetes in the young study, Diabetes 65 (7) (2016) 1988–1995.
[145] Y.-X. Zhao, X.-W. Chen, Diabetes and risk of glaucoma: systematic review and a Meta-analysis of prospective cohort studies, Int. J. Ophthalmol. 10 (2017) 1430.
[146] R.R. Huxley, K.B. Filion, S. Konety, A. Alonso, Meta-analysis of cohort and case–control studies of type 2 diabetes mellitus and risk of atrial fibrillation, Am. J. Cardiol. 108 (2011) 56–62.
[147] M. Li, Y. Fan, X. Zhang, W. Hou, Z. Tang, Fruit and vegetable intake and risk of type 2 diabetes mellitus: meta-analysis of prospective cohort studies, BMJ Open 4 (2014), e005497.
[148] A.V. Ardisson Korat, W.C. Willett, F.B. Hu, Diet, lifestyle, and genetic risk factors for type 2 diabetes: a review from the Nurses' Health Study, Nurses' Health Study 2, and Health Professionals' Follow-up Study, Current nutrition reports 3 (2014) 345–354.
[149] A. King, J. Bowe, Animal models for diabetes: understanding the pathogenesis and finding new treatments, Biochem. Pharmacol. 99 (2016) 1–10.
[150] M. Von Herrath, G.T. Nepom, Animal models of human type 1 diabetes, Nat. Immunol. 10 (2009) 129–132.
[152] P.C. Chandrasekera, Of rodents and men: species-specific glucose regulation and type 2 diabetes research, ALTEX (2014) 157–176.
[153] K.H. Benam, S. Dauth, B. Hassell, A. Herland, A. Jain, K.J. Jang, K. Karalis, H.J. Kim, L. MacQueen, R. Mahmoodian, S. Musah, Y.S. Torisawa, A.D. van der Meer, R. Villenave, M. Yadid, K.K. Parker, D.E. Ingber, Engineered in vitro disease models, Annu. Rev. Pathol. 10 (2015) 195–262.
[154] J.N. Weinstein, Drug discovery: cell lines battle cancer, Nature 483 (2012) 544–545.
[155] F. Martín, A. Sánchez-Gilabert, M. Tristán-Manzano, K. Benabdellah, Stem Cells for Modeling Human Disease Pluripotent Stem Cells-From the Bench to the Clinic, InTech, 2016.
[156] L.N. Randolph, Y. Jiang, X. Lian, Stem Cell Engineering and Differentiation for Disease Modeling and Cell-Based Therapies, Elsevier, 2017.
[157] N. Allum, A. Allansdottir, G. Gaskell, J. Hampel, J. Jackson, A. Moldovan, S. Priest, S. Stares, P. Stoneman, Religion and the public ethics of stem-cell research: attitudes in Europe, Canada and the United States, PLoS One 12 (2017), e0176274.
[158] B. Nashun, P.W. Hill, P. Hajkova, Reprogramming of cell fate: epigenetic memory and the erasure of memories past, EMBO J. 34 (2015) 1296–1308.
[159] M.C. Marchetto, G.W. Yeo, O. Kainohana, M. Marsala, F.H. Gage, A.R. Muotri, Transcriptional signature and memory retention of human-induced pluripotent stem cells, PLoS One 4 (2009), e7076.
[160] R. Lister, M. Pelizzola, Y.S. Kida, R.D. Hawkins, J.R. Nery, G. Hon, J. Antosiewicz-Bourget, R. O'Malley, R. Castanon, S. Klugman, Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells, Nature 471 (2011) 68–73.
[161] K. Saha, R. Jaenisch, Technical challenges in using human induced pluripotent stem cells to model disease, Cell Stem Cell 5 (2009) 584–595.
[162] P. Horvath, N. Aulner, M. Bickle, A.M. Davies, E. Del Nery, D. Ebner, M.C. Montoya, P. Östling, V. Pietiäinen, L.S. Price, Screening out irrelevant cell-based models of disease, Nat. Rev. Drug Discov. 15 (2016) 751–769.
[163] J. Stepniewski, N. Kachamakova-Trojanowska, D. Ogrocki, M. Szopa, M. Matlok, M. Beilharz, G. Dyduch, M. Malecki, A. Józkowicz, J. Dulak, Induced pluripotent stem cells as a model for diabetes investigation, Sci. Rep. 5 (2015).
[164] Y.C. Kudva, S. Ohmine, L.V. Greder, J.R. Dutton, A. Armstrong, J.G. De Lamo, Y.K. Khan, T. Thatava, M. Hasegawa, N. Fusaki, J.M.W. Slack, Y. Ikeda, Transgene-free disease-specific induced pluripotent stem cells from patients with type 1 and type 2 diabetes, Stem Cells Transl. Med. 1 (2012) 451–461.
[165] J.P. Whitehead, M.A. Soos, R. Jackson, V. Tasic, M. Kocova, S. O'Rahilly, Multiple molecular mechanisms of insulin receptor dysfunction in a patient with donohue syndrome, Diabetes 47 (1998) 1362.
[166] A.M. Burkart, K. Tan, L. Warren, S. Iovino, K.J. Hughes, C.R. Kahn, M.E. Patti, Insulin resistance in human iPS cells reduces mitochondrial size and function, Sci. Rep. 6 (2016), 22788.
[167] S. Iovino, A.M. Burkart, K. Kriauciunas, L. Warren, K.J. Hughes, M. Molla, Y.-K. Lee, M.-E. Patti, C.R. Kahn, Genetic insulin resistance is a potent regulator of gene expression and proliferation in human iPS cells, Diabetes 63 (2014) 4130–4142.
[168] S.N. Bhatia, D.E. Ingber, Microfluidic organs-on-chips, Nat. Biotechnol. 32 (2014) 760–772.
[169] R.G. Harrison, M.J. Greenman, F.P. Mall, C.M. Jackson, Observations of the living developing nerve fiber, Anat. Rec. 1 (1907) 116–128.
[170] S. Breslin, L. O'Driscoll, Three-dimensional cell culture: the missing link in drug discovery, Drug Discov. Today 18 (2013) 240–249.
[171] 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.
[172] A.K. Capulli, K. Tian, N. Mehandru, A. Bukhta, S.F. Choudhury, M. Suchyta, K.K. Parker, Approaching the in vitro clinical trial: engineering organs on chips, Lab Chip 14 (2014) 3181–3186.
[173] D. Huh, G.A. Hamilton, D.E. Ingber, From 3D cell culture to organs-on-chips, Trends Cell Biol. 21 (2011) 745–754.
[174] M.W. Tibbitt, K.S. Anseth, Hydrogels as extracellular matrix mimics for 3D Cell Culture, Biotechnol. Bioeng. 103 (2009) 655–663.
[175] A. Fatehullah, S.H. Tan, N. Barker, Organoids as an in vitro model of human development and disease, Nat. Cell Biol. 18 (2016) 246.
[176] H. Clevers, Modeling development and disease with organoids, Cell 165 (2016) 1586–1597.
[177] J. Rogal, C. Probst, P. Loskill, Integration concepts for multi-organ chips how to maintain flexibility?! Future Science OA 3 (2) (2017) FSO180.
[178] J.S. Kaddis, B.J. Olack, J. Sowinski, J. Cravens, J.L. Contreras, J.C. Niland, Human pancreatic islets and diabetes research, JAMA 301 (2009) 1580–1587.
[179] M. Skelin, M. Rupnik, A. Cencic, Pancreatic beta cell lines and their applications in diabetes mellitus research, ALTEX 27 (2010) 105–113.
[180] E. Bader, A. Migliorini, M. Gegg, N. Moruzzi, J. Gerdes, S.S. Roscioni, M. Bakhti, E. Brandl, M. Irmler, J. Beckers, Identification of proliferative and mature β-cells in the islets of Langerhans, Nature 535 (2016) 430–434.
[181] P. Ravassard, Y. Hazhouz, S. Pechberty, E. Bricout-Neveu, M. Armanet, P. Czernichow, R. Scharfmann, A genetically engineered human pancreatic β cell line exhibiting glucose-inducible insulin secretion, J. Clin. Invest. 121 (2011) 3589.
[182] R. Scharfmann, S. Pechberty, Y. Hazhouz, M. Von Bülow, E. Bricout-Neveu, M. Grenier-Godard, F. Guez, L. Rachdi, M. Lohmann, P. Czernichow, Development of a conditionally immortalized human pancreatic β cell line, J. Clin. Invest. 124 (2014) 2087.
[183] M. Benazra, M.-J. Lecomte, C. Colace, A. Müller, C. Machado, S. Pechberty, E. Bricout-Neveu, M. Grenier-Godard, M. Solimena, R. Scharfmann, A human beta cell line with drug inducible excision of immortalizing transgenes, Molecular metabolism 4 (2015) 916–925.
[184] L.S.W. Loo, H.H. Lau, J.B. Jasmen, C.S. Lim, A.K.K. Teo, An arduous journey from human pluripotent stem cells to functional pancreatic β-cells, Diabetes. Obes. Metab. 20 (1) (2018) 3–13.
[185] S. Assady, G. Maor, M. Amit, J. Itskovitz-Eldor, K.L. Skorecki, M. Tzukerman, Insulin production by human embryonic stem cells, Diabetes 50 (2001) 1691–1697.
[186] X. Xu, B. Kahan, A. Forgianni, P. Jing, L. Jacobson, V. Browning, N. Treff, J. Odorico, Endoderm and pancreatic islet lineage differentiation from human embryonic stem cells, Cloning Stem Cells 8 (2006) 96–107.
[187] H. Segev, B. Fishman, A. Ziskind, M. Shulman, J. Itskovitz-Eldor, Differentiation of human embryonic stem cells into insulin-producing clusters, Stem Cells 22 (2004) 265–274.
[188] B.W. Phillips, H. Hentze, W.L. Rust, Q.-P. Chen, H. Chipperfield, E.-K. Tan, S. Abraham, A. Sadasivam, P.L. Soong, S.T. Wang, Directed differentiation of human embryonic stem cells into the pancreatic endocrine lineage, Stem Cells Dev. 16 (2007) 561–578.
[189] 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.
[190] J. Jiang, M. Au, K. Lu, A. Eshpeter, G. Korbutt, G. Fisk, A.S. Majumdar, Generation of insulin-producing islet-like clusters from human embryonic stem cells, Stem Cells 25 (2007) 1940–1953.
[191] W. Jiang, Y. Shi, D. Zhao, S. Chen, J. Yong, J. Zhang, T. Qing, X. Sun, P. Zhang, M. Ding, In vitro derivation of functional insulin-producing cells from human embryonic stem cells, Cell Res. 17 (2007) 333.
[192] D. Zhang, W. Jiang, M. Liu, X. Sui, X. Yin, S. Chen, Y. Shi, H. Deng, Highly efficient differentiation of human ES cells and iPS cells into mature pancreatic insulin-producing cells, Cell Res. 19 (2009) 429.
[193] J. Shim, S. Kim, D. Woo, S. Kim, C. Oh, R. McKay, J. Kim, Directed differentiation of human embryonic stem cells towards a pancreatic cell fate, Diabetologia 50 (2007) 1228–1238.
[194] E. Kroon, L.A. Martinson, K. Kadoya, A.G. Bang, O.G. Kelly, S. Eliazer, H. Young, M. Richardson, N.G. Smart, J. Cunningham, Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo, Nat. Biotechnol. 26 (2008) 443.
[195] A. Rezania, J.E. Bruin, M.J. Riedel, M. Mojibian, A. Asadi, J. Xu, R. Gauvin, K. Narayan, F. Karanu, J.J. O'Neil, Maturation of human embryonic stem cell–derived pancreatic progenitors into functional islets capable of treating pre-existing diabetes in mice, Diabetes 61 (2012) 2016–2029.
[196] J.E. Bruin, A. Rezania, J. Xu, K. Narayan, J.K. Fox, J.J. O'Neil, T.J. Kieffer, Maturation and function of human embryonic stem cell-derived pancreatic progenitors in macroencapsulation devices following transplant into mice, Diabetologia 56 (2013) 1987–1998.
[197] A. Rezania, J.E. Bruin, J. Xu, K. Narayan, J.K. Fox, J.J. O'Neil, T.J. Kieffer, Enrichment of human embryonic stem cell-derived NKX6. 1-expressing pancreatic progenitor cells accelerates the maturation of insulin-secreting cells in vivo, Stem Cells 31 (2013) 2432–2442.

[198] F.W. Pagliuca, J.R. Millman, M. Gürtler, M. Segel, A. Van Dervort, J.H. Ryu, Q.P. Peterson, D. Greiner, D.A. Melton, Generation of functional human pancreatic β cells in vitro, Cell 159 (2014) 428–439.
[199] A. Rezania, J.E. Bruin, P. Arora, A. Rubin, I. Batushansky, A. Asadi, S. O'dwyer, N. Quiskamp, M. Mojibian, T. Albrecht, Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells, Nat. Biotechnol. 32 (2014) 1121–1133.
[200] O.G. Kelly, M.Y. Chan, L.A. Martinson, K. Kadoya, T.M. Ostertag, K.G. Ross, M. Richardson, M.K. Carpenter, K.A. D'amour, E. Kroon, Cell-surface markers for the isolation of pancreatic cell types derived from human embryonic stem cells, Nat. Biotechnol. 29 (2011) 750–756.
[201] H.A. Russ, A.V. Parent, J.J. Ringler, T.G. Hennings, G.G. Nair, M. Shveygert, T. Guo, S. Puri, L. Haataja, V. Cirulli, Controlled induction of human pancreatic progenitors produces functional beta-like cells in vitro, EMBO J. 34 (2015) 1759–1772.
[202] R. Bhonde, R. Shukla, M. Kanitkar, R. Shukla, Isolated islets in diabetes research, Indian J. Med. Res. 125 (2007) 425.
[203] B.M. Schmied, A. Ulrich, H. Matsuzaki, S.K. Batra, P.M. Pour, X. Ding, T.E. Adrian, C. Ricordi, M.P. Moyer, Maintenance of human islets in long term culture, Differentiation 66 (2000) 173–180.
[204] S. Williams, T. Schwasinger-Schmidt, D. Zamierowski, L. Stehno-Bittel, Diffusion into human islets is limited to molecules below 10kDa, Tissue Cell 44 (2012) 332–341.
[205] J. Hilderink, S. Spijker, F. Carlotti, L. Lange, M. Engelse, C. Blitterswijk, E. Koning, M. Karperien, A. Apeldoorn, Controlled aggregation of primary human pancreatic islet cells leads to glucose-responsive pseudoislets comparable to native islets, J. Cell. Mol. Med. 19 (2015) 1836–1846.
[206] R. Zuellig, G. Cavallari, P. Gerber, O. Tschopp, G. Spinas, W. Moritz, R. Lehmann, Improved physiological properties of gravity-enforced reassembled rat and human pancreatic pseudo-islets, J. Tissue Eng. Regen. Med. 11 (2017) 109–120.
[207] Y. Ichihara, R. Utoh, M. Yamada, T. Shimizu, Y. Uchigata, Size effect of engineered islets prepared using microfabricated wells on islet cell function and arrangement, Heliyon 2 (2016), e00129.
[208] M.-J. Lecomte, S. Pechberty, C. Machado, S. Da Barroca, P. Ravassard, R. Scharfmann, P. Czernichow, B. Duvillié, Aggregation of engineered human β-cells into pseudoislets: insulin secretion and gene expression profile in normoxic and hypoxic milieu, Cell Med. 8 (2016) 99–112.
[209] K. Ramachandran, S.J. Williams, H.-H. Huang, L. Novikova, L. Stehno-Bittel, Engineering islets for improved performance by optimized reaggregation in a micromold, Tissue Eng. A 19 (2012) 604–612.
[210] B. Gao, L. Wang, S. Han, B. Pingguan-Murphy, X. Zhang, F. Xu, Engineering of microscale three-dimensional pancreatic islet models in vitro and their biomedical applications, Crit. Rev. Biotechnol. 36 (2016) 619–629.
[211] L.M. Weber, J. He, B. Bradley, K. Haskins, K.S. Anseth, PEG-based hydrogels as an in vitro encapsulation platform for testing controlled β-cell microenvironments, Acta Biomater. (2) (2006) 1–8.
[212] E. O'sullivan, A. Johnson, A. Omer, J. Hollister-Lock, S. Bonner-Weir, C. Colton, G. Weir, Rat islet cell aggregates are superior to islets for transplantation in microcapsules, Diabetologia 53 (2010) 937–945.
[213] A.D. Mendelsohn, D.A. Bernards, R.D. Lowe, T.A. Desai, Patterning of mono-and multilayered pancreatic β-cell clusters, Langmuir 26 (2010) 9943–9949.
[214] J.M. Karp, J. Yeh, G. Eng, J. Fukuda, J. Blumling, K.-Y. Suh, J. Cheng, A. Mahdavi, J. Borenstein, R. Langer, Controlling size, shape and homogeneity of embryoid bodies using poly (ethylene glycol) microwells, Lab Chip 7 (2007) 786–794.
[215] T.H. Hraha, A.B. Bernard, L.M. Nguyen, K.S. Anseth, R.K. Benninger, Dimensionality and size scaling of coordinated Ca 2+ dynamics in MIN6 β-cell clusters, Biophys. J. 106 (2014) 299–309.
[216] M. Shinohara, H. Kimura, K. Montagne, K. Komori, T. Fujii, Y. Sakai, Combination of microwell structures and direct oxygenation enables efficient and size-regulated aggregate formation of an insulin-secreting pancreatic β-cell line, Biotechnol. Prog. 30 (2014) 178–187.
[217] J. Amin, K. Ramachandran, S.J. Williams, A. Lee, L. Novikova, L. Stehno-Bittel, A simple, reliable method for high-throughput screening for diabetes drugs using 3D β-cell spheroids, J. Pharmacol. Toxicol. Methods 82 (2016) 83–89.
[218] I. Akerman, Z. Tu, A. Beucher, D.M. Rolando, C. Sauty-Colace, M. Benazra, N. Nakic, J. Yang, H. Wang, L. Pasquali, Human pancreatic β cell lncRNAs control cell-specific regulatory networks, Cell Metab. 25 (2017) 400–411.
[219] M. Krishnamurthy, J. Li, M. Al-Masri, R. Wang, Expression and function of αβ1 integrins in pancretic beta (INS-1) cells, J. Cell Commun. Signal. 2 (2008) 67–79.
[220] G.J. Szebeni, Z. Tancos, L.Z. Feher, R. Alfoldi, J. Kobolak, A. Dinnyes, L.G. Puskas, Real architecture For 3D Tissue (RAFT™) culture system improves viability and maintains insulin and glucagon production of mouse pancreatic islet cells, Cytotechnology 69 (2017) 359–369.
[221] M. Riopel, R. Wang, Collagen matrix support of pancreatic islet survival and function, Front. Biosci. (2014) 19.
[222] J.T. Daoud, M.S. Petropavlovskaia, J.M. Patapas, C.E. Degrandpré, R.W. DiRaddo, L. Rosenberg, M. Tabrizian, Long-term in vitro human pancreatic islet culture using three-dimensional microfabricated scaffolds, Biomaterials 32 (2011) 1536–1542.
[223] Z. Li, H. Sun, J. Zhang, H. Zhang, F. Meng, Z. Cui, Development of in vitro 3D Tissueflex® islet model for diabetic drug efficacy testing, PLoS One 8 (2013), e72612.
[224] G. Orive, E. Santos, D. Poncelet, R.M. Hernández, J.L. Pedraz, L.U. Wahlberg, P. De Vos, D. Emerich, Cell encapsulation: technical and clinical advances, Trends Pharmacol. Sci. 36 (2015) 537–546.
[225] L. Shang, H. Hua, K. Foo, H. Martinez, K. Watanabe, M. Zimmer, D.J. Kahler, M. Freeby, W. Chung, C. LeDuc, β-cell dysfunction due to increased ER stress in a stem cell model of Wolfram syndrome, Diabetes 63 (2014) 923–933.
[226] S.G. Yabe, N. Iwasaki, K. Yasuda, T.S. Hamazaki, M. Konno, S. Fukuda, F. Takeda, M. Kasuga, H. Okochi, Establishment of maturity-onset diabetes of the young-induced pluripotent stem cells from a Japanese patient, J. Diabetes Investig. 6 (2015) 543–547.
[227] A.K. Teo, R. Windmueller, B.B. Johansson, E. Dirice, P.R. Njolstad, E. Tjora, H. Raeder, R.N. Kulkarni, Derivation of human induced pluripotent stem cells from patients with maturity onset diabetes of the young, J. Biol. Chem. 288 (2013) 5353–5356.
[228] A.K.K. Teo, H.H. Lau, I.A. Valdez, E. Dirice, E. Tjora, H. Raeder, R.N. Kulkarni, Early developmental perturbations in a human stem cell model of MODY5/HNF1B pancreatic hypoplasia, Stem Cell Reports 6 (2016) 357–367.
[229] S.S. Fajans, Maturity-onset diabetes of the young (MODY), Diabetes Metab. Res. Rev. 5 (1989) 579–606.
[230] J.S. Mohammed, Y. Wang, T.A. Harvat, J. Oberholzer, D.T. Eddington, Microfluidic device for multimodal characterization of pancreatic islets, Lab Chip 9 (2009) 97–106.
[231] X. Li, J.C. Brooks, J. Hu, K.I. Ford, C.J. Easley, 3D-templated, fully automated microfluidic input/output multiplexer for endocrine tissue culture and secretion sampling, Lab Chip 17 (2017) 341–349.
[232] J.C. Brooks, K.I. Ford, D.H. Holder, M.D. Holtan, C.J. Easley, Macro-to-micro interfacing to microfluidic channels using 3D-printed templates: application to time-resolved secretion sampling of endocrine tissue, Analyst 141 (2016) 5714–5721.
[233] Y. Xing, M. Nourmohammadzadeh, J.E. Elias, M. Chan, Z. Chen, J.J. McGarrigle, J. Oberholzer, Y. Wang, A pumpless microfluidic device driven by surface tension for pancreatic islet analysis, Biomed. Microdevices 18 (2016) 80.
[234] T. Schulze, K. Mattern, E. Früh, L. Hecht, I. Rustenbeck, A. Dietzel, A 3D microfluidic perfusion system made from glass for multiparametric analysis of stimulus-secretioncoupling in pancreatic islets, Biomed. Microdevices 19 (2017) 47.
[235] S.H. Lee, S. Hong, J. Song, B. Cho, E.J. Han, S. Kondapavulur, D. Kim, L.P. Lee, Microphysiological analysis platform of pancreatic islet β-cell spheroids, Adv. Healthc. Mater. 7 (2018), 1701111.
[236] M. Atkinson, J. Bluestone, G. Eisenbarth, M. Hebrok, K. Herold, D. Accili, M. Pietropaolo, P. Arvan, M. Von Herrath, D. Markel, How does type 1 diabetes develop? The notion of homicide or β-cell suicide revisited, Diabetes 60 (2011) 1370–1379 Diabetes, 61 (2012).
[237] N. Gopalakrishnan, R. Hannam, G. Casoni, D. Barriet, J. Ribe, M. Haug, Ø. Halaas, Infection and immunity on a chip: a compartmentalised microfluidic platform to monitor immune cell behaviour in real time, Lab Chip 15 (2015) 1481–1487.
[238] S.K. Kim, W.K. Moon, J.Y. Park, H. Jung, Inflammatory mimetic microfluidic chip by immobilization of cell adhesion molecules for T cell adhesion, Analyst 137 (2012) 4062–4068.
[239] P. Sundd, E. Gutierrez, E.K. Koltsova, Y. Kuwano, S. Fukuda, M.K. Pospieszalska, A. Groisman, K. Ley, Slings/'enable neutrophil rolling at high shear, Nature 488 (2012) 399–403.
[240] B. Zhang, C. Peticone, S.K. Murthy, M. Radisic, A standalone perfusion platform for drug testing and target validation in micro-vessel networks, Biomicrofluidics 7 (2013), 044125.
[241] S. Srigunapalan, C. Lam, A.R. Wheeler, C.A. Simmons, A microfluidic membrane device to mimic critical components of the vascular microenvironment, Biomicrofluidics 5 (2011), 013409.
[242] K.H. Benam, R. Villenave, C. Lucchesi, A. Varone, C. Hubeau, H.-H. Lee, S.E. Alves, M. Salmon, T.C. Ferrante, J.C. Weaver, Small airway-on-a-chip enables analysis of human lung inflammation and drug responses in vitro, Nat. Methods 13 (2016) 151–157.
[243] H.J. Kim, H. Li, J.J. Collins, D.E. Ingber, Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip, Proc. Natl. Acad. Sci. 113 (2016) E7–E15.
[244] M. Gröger, K. Rennert, B. Giszas, E. Weiß, J. Dinger, H. Funke, M. Kiehntopf, F.T. Peters, A. Lupp, M. Bauer, Monocyte-induced recovery of inflammation-associated hepatocellular dysfunction in a biochip-based human liver model, Sci. Rep. 6 (2016), 21868.
[245] P. Godoy, N.J. Hewitt, U. Albrecht, M.E. Andersen, N. Ansari, S. Bhattacharya, J.G. Bode, J. Bolleyn, C. Borner, J. Böttger, 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.P. Goldring, M.J. Gómez-Lechón, G.M.M. Groothuis, L. Gustavsson, C. Guyot, D. Hallifax, S. Hammad, A. Hayward, D. Häussinger, C. Hellerbrand, P. Hewitt, S. Hoehme, H.-G. Holzhütter, 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.K. Stelzer, B. Stieger, R. Stöber, 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.
[246] K. Zeilinger, N. Freyer, G. Damm, D. Seehofer, F. Knöspel, Cell sources for in vitro human liver cell culture models, Exp. Biol. Med. 241 (2016) 1684–1698.
[247] M.D. Davidson, M. Lehrer, S.R. Khetani, Hormone and drug-mediated modulation of glucose metabolism in a microscale model of the human liver, Tissue Eng. Part C 21 (2014) 716–725.
[248] S. Wilkening, F. Stahl, A. Bader, Comparison of primary human hepatocytes and hepatoma cell line HepG2 with regard to their biotransformation properties, Drug Metab. Dispos. 31 (2003) 1035–1042.

[249] T.B. Andersson, K.P. Kanebratt, J.G. Kenna, The HepaRG cell line: a unique in vitro tool for understanding drug metabolism and toxicology in human, Expert Opin. Drug Metab. Toxicol. 8 (2012) 909–920.
[250] R.E. Schwartz, H.E. Fleming, S.R. Khetani, S.N. Bhatia, Pluripotent stem cell-derived hepatocyte-like cells, Biotechnol. Adv. 32 (2014) 504–513.
[251] Z. Hannoun, C. Steichen, N. Dianat, A. Weber, A. Dubart-Kupperschmitt, The potential of induced pluripotent stem cell derived hepatocytes, J. Hepatol. 65 (2016) 182–199.
[252] C. Lin, K.R. Ballinger, S.R. Khetani, The application of engineered liver tissues for novel drug discovery, Expert Opin. Drug Discovery 10 (2015) 519–540.
[253] F. Pius, J.B. Patrick, R. Bernd, Liver-based in vitro technologies for drug biotransformation studies – a review, Curr. Drug Metab. 13 (2012) 215–224.
[254] A.E.M. Vickers, P. Bentley, R.L. Fisher, Consequences of mitochondrial injury induced by pharmaceutical fatty acid oxidation inhibitors is characterized in human and rat liver slices, Toxicol. in Vitro 20 (2006) 1173–1182.
[255] V. Starokozhko, S. Vatakuti, B. Schievink, M.T. Merema, A. Asplund, J. Synnergren, A. Aspegren, G.M.M. Groothuis, Maintenance of drug metabolism and transport functions in human precision-cut liver slices during prolonged incubation for 5 days, Arch. Toxicol. 91 (2017) 2079–2092.
[256] M.D. Davidson, K.R. Ballinger, S.R. Khetani, Long-term exposure to abnormal glucose levels alters drug metabolism pathways and insulin sensitivity in primary human hepatocytes, Sci. Rep. 6 (2016), 28178.
[257] C. Lin, S.R. Khetani, Micropatterned Co-Cultures of Human Hepatocytes and Stromal Cells for the Assessment of Drug Clearance and Drug-Drug Interactions Current Protocols in Toxicology, John Wiley & Sons, Inc., 2017
[258] 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.
[259] B.R. Ware, S.R. Khetani, Micropatterned co-cultures of induced pluripotent stem cell-derived hepatocytes and stromal cells for drug toxicity studies, in: M. Clements, L. Roquemore (Eds.), Stem Cell-Derived Models in Toxicology, Springer, New York 2017, pp. 311–334.
[260] N.S. Bhise, V. Manoharan, S. Massa, A. Tamayol, M. Ghaderi, M. Miscuglio, Q. Lang, Y. Shrike Zhang, S.R. Shin, G. Calzone, N. Annabi, T.D. Shupe, C.E. Bishop, A. Atala, M.R. Dokmeci, A. Khademhosseini, A liver-on-a-chip platform with bioprinted hepatic spheroids, Biofabrication 8 (2016), 014101.
[261] L.A. Vernetti, N. Senutovitch, R. Boltz, R. DeBiasio, T.Y. Shun, A. Gough, D.L. Taylor, A human liver microphysiology platform for investigating physiology, drug safety, and disease models, Exp Biol Med (Maywood) 241 (2016) 101–114.
[262] 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.
[263] 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.
[264] 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.
[265] 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.
[266] A.A. Banaeiyan, J. Theobald, J. Paukštyte, S. Wölfl, C.B. Adiels, M. Goksör, Design and fabrication of a scalable liver-lobule-on-a-chip microphysiological platform, Biofabrication 9 (2017), 015014.
[267] Y.-C. Toh, T.C. Lim, D. Tai, G. Xiao, D. van Noort, H. Yu, A microfluidic 3D hepatocyte chip for drug toxicity testing, Lab Chip 9 (2009) 2026–2035.
[268] K. Domansky, W. Inman, J. Serdy, A. Dash, M.H.M. Lim, L.G. Griffith, Perfused multiwell plate for 3D liver tissue engineering, Lab Chip 10 (2010) 51–58.
[269] Y. Nakao, H. Kimura, Y. Sakai, T. Fujii, Bile canaliculi formation by aligning rat primary hepatocytes in a microfluidic device, Biomicrofluidics 5 (2011), 022212.
[270] A. Schepers, C. Li, A. Chhabra, B.T. Seney, S. Bhatia, Engineering a perfusable 3D human liver platform from iPS cells, Lab Chip 16 (2016) 2644–2653.
[271] K.M. Tharp, A.K. Jha, J. Kraiczy, A. Yesian, G. Karateev, R. Sinisi, E.A. Dubikovskaya, K.E. Healy, A. Stahl, Matrix-assisted transplantation of functional beige adipose tissue, Diabetes 64 (2015) 3713–3724.
[272] B. Huber, A. Link, K. Linke, S.A. Gehrke, M. Winnefeld, P.J. Kluger, Integration of mature adipocytes to build-up a functional three-layered full-skin equivalent, Tissue Eng. Part C Methods 22 (2016) 756–764.
[273] A. Ahluwalia, A. Misto, F. Vozzi, C. Magliaro, G. Mattei, M.C. Marescotti, A. Avogaro, E. Iori, Systemic and vascular inflammation in an in-vitro model of central obesity, PLoS One 13 (2018), e0192824.
[274] S. Virtue, A. Vidal-Puig, Adipose tissue expandability, lipotoxicity and the Metabolic Syndrome–an allostatic perspective, Biochim. Biophys. Acta 1801 (2010) 338–349.
[275] C.T. Gomillion, K.J. Burg, Stem cells and adipose tissue engineering, Biomaterials 27 (2006) 6052–6063.
[276] D. Taura, M. Noguchi, M. Sone, K. Hosoda, E. Mori, Y. Okada, K. Takahashi, K. Homma, N. Oyamada, M. Inuzuka, T. Sonoyama, K. Ebihara, N. Tamura, H. Itoh, H. Suemori, N. Nakatsuji, H. Okano, S. Yamanaka, K. Nakao, Adipogenic differentiation of human induced pluripotent stem cells: comparison with that of human embryonic stem cells, FEBS Lett. 583 (2009) 1029–1033.
[277] X. Li, C.J. Easley, Microfluidic systems for studying dynamic function of adipocytes and adipose tissue, Anal. Bioanal. Chem. 410(3), 2018, 791-800.
[278] A.-M. Rodriguez, C. Elabd, F. Delteil, J. Astier, C. Vernochet, P. Saint-Marc, J. Guesnet, A. Guezennec, E.-Z. Amri, C. Dani, G. Ailhaud, Adipocyte differentiation of multipotent cells established from human adipose tissue, Biochem. Biophys. Res. Commun. 315 (2004) 255–263.
[279] A.-C. Guénantin, N. Briand, E. Capel, F. Dumont, R. Morichon, C. Provost, F. Stillitano, D. Jeziorowska, J.-P. Siffroi, R.J. Hajjar, B. Fève, J.-S. Hulot, P. Collas, J. Capeau, C. Vigouroux, Functional human beige adipocytes from induced pluripotent stem cells, Diabetes 66 (2017) 1470–1478.
[280] T. Mohsen-Kanson, A.L. Hafner, B. Wdziekonski, Y. Takashima, P. Villageois, A. Carriere, M. Svensson, C. Bagnis, B. Chignon-Sicard, P.A. Svensson, L. Casteilla, A. Smith, C. Dani, Differentiation of human induced pluripotent stem cells into brown and white adipocytes: role of Pax3, Stem Cells 32 (2014) 1459–1467.
[281] A.L. Hafner, C. Dani, Human induced pluripotent stem cells: a new source for brown and white adipocytes, World J Stem Cells 6 (2014) 467–472.
[282] I. Barbagallo, G.L. Volti, F. Galvano, G. Tettamanti, F.R. Pluchinotta, S. Bergante, L. Vanella, Diabetic human adipose tissue-derived mesenchymal stem cells fail to differentiate in functional adipocytes, Exp. Biol. Med. 242 (2017) 1079–1085.
[283] B. Huber, A.C. Volz, P.J. Kluger, Understanding the effects of mature adipocytes and endothelial cells on fatty acid metabolism and vascular tone in physiological fatty tissue for vascularized adipose tissue engineering, Cell Tissue Res. 362 (2015) 269–279.
[284] J.H. Choi, J.M. Gimble, K. Lee, K.G. Marra, J.P. Rubin, J.J. Yoo, G. Vunjak-Novakovic, D.L. Kaplan, Adipose tissue engineering for soft tissue regeneration, Tissue Eng Part B Rev 16 (2010) 413–426.
[285] K. Wittmann, S. Dietl, N. Ludwig, O. Berberich, C. Hoefner, K. Storck, T. Blunk, P. Bauer-Kreisel, Engineering vascularized adipose tissue using the stromal-vascular fraction and fibrin hydrogels, Tissue Eng. A 21 (2015) 1343–1353.
[286] R.D. Abbott, F.E. Borowsky, C.A. Alonzo, A. Zieba, I. Georgakoudi, D.L. Kaplan, Variability in responses observed in human white adipose tissue models, J. Tissue Eng. Regen. Med. 12 (3) (2018) 840–847.
[287] R.D. Abbott, E.P. Kimmerling, D.M. Cairns, D.L. Kaplan, Silk as a biomaterial to support long-term three-dimensional tissue cultures, ACS Appl. Mater. Interfaces 8 (2016) 21861–21868.
[288] R.D. Abbott, R.Y. Wang, M.R. Reagan, Y. Chen, F.E. Borowsky, A. Zieba, K.G. Marra, J.P. Rubin, I.M. Ghobrial, D.L. Kaplan, The use of silk as a scaffold for mature, sustainable unilocular adipose 3D tissue engineered systems, Adv. Healthc. Mater. 5 (2016) 1667–1677.
[289] R.D. Abbott, W.K. Raja, R.Y. Wang, J.A. Stinson, D.L. Glettig, K.A. Burke, D.L. Kaplan, Long term perfusion system supporting adipogenesis, Methods 84 (2015) 84–89.
[290] P.A. Turner, Y. Tang, S.J. Weiss, A.V. Janorkar, Three-dimensional spheroid cell model of in vitro adipocyte inflammation, Tissue Eng Part A 21 (2015) 1837–1847.
[291] I. Zagotta, E.Y. Dimova, K.M. Debatin, M. Wabitsch, T. Kietzmann, P. Fischer-Posovszky, Obesity and inflammation: reduced cytokine expression due to resveratrol in a human in vitro model of inflamed adipose tissue, Front. Pharmacol. 6 (2015) 79.
[292] K. Viravaidya, M.L. Shuler, Incorporation of 3T3-L1 cells to mimic bioaccumulation in a microscale cell culture analog device for toxicity studies, Biotechnol. Prog. 20 (2004) 509–597.
[293] P. Loskill, T. Sezhian, K.M. Tharp, F.T. Lee-Montiel, S. Jeeawoody, W.M. Reese, P.H. Zushin, A. Stahl, K.E. Healy, WAT-on-a-chip: a physiologically relevant microfluidic system incorporating white adipose tissue, Lab Chip 17 (2017) 1645–1654.
[294] C. Moraes, J.M. Labuz, B.M. Leung, M. Inoue, T.H. Chun, S. Takayama, On being the right size: scaling effects in designing a human-on-a-chip, Integr Biol (Camb) 5 (2013) 1149–1161.
[295] J. Wu, Thermogenic Fat: Methods and Protocols. , Springer, 2017.
[296] L.A. Godwin, J.C. Brooks, L.D. Hoepfner, D. Wanders, R.L. Judd, C.J. Easley, A microfluidic interface for the culture and sampling of adiponectin from primary adipocytes, Analyst 140 (2015) 1019–1025.
[297] T.H. Qazi, D.J. Mooney, M. Pumberger, S. Geissler, G.N. Duda, Biomaterials based strategies for skeletal muscle tissue engineering: existing technologies and future trends, Biomaterials 53 (2015) 502–521.
[298] J. Stern-Straeter, F. Riedel, G. Bran, K. Hörmann, U.R. Gössler, Advances in skeletal muscle tissue engineering, In Vivo 21 (2007) 435–444.
[299] A.S.T. Smith, J. Davis, G. Lee, D.L. Mack, D.H. Kim, Muscular dystrophy in a dish: engineered human skeletal muscle mimetics for disease modeling and drug discovery, Drug Discov. Today 21 (2016) 1387–1398.
[300] H. Bethany, M. Reza, M. Pouya, K. Wasim, The regulation of differentiation of mesenchymal stem-cells into skeletal muscle a look at signalling molecules involved in myogenesis, Curr. Stem Cell Res. Ther. 12 (2017) 1–24.
[301] R. Darabi, K. Gehlbach, R.M. Bachoo, S. Kamath, M. Osawa, K.E. Kamm, M. Kyba, R.C.R. Perlingeiro, Functional skeletal muscle regeneration from differentiating embryonic stem cells, Nat. Med. 14 (2008) 134.
[302] T.A. Partridge, J.E. Morgan, G.R. Coulton, E.P. Hoffman, L.M. Kunkel, Conversion of mdx myofibres from dystrophin-negative to -positive by injection of normal myoblasts, Nature 337 (1989) 176.
[303] R. Darabi, W. Pan, D. Bosnakovski, J. Baik, M. Kyba, R.C.R. Perlingeiro, Functional myogenic engraftment from mouse iPS cells, Stem Cell Rev. Rep. 7 (2011) 948–957.
[304] Y. Miyagoe-Suzuki, S.i. Takeda, Skeletal muscle generated from induced pluripotent stem cells – induction and application, World J. Stem Cells 9 (2017) 89–97.
[305] M. Shelton, A. Kocharyan, J. Liu, I.S. Skerjanc, W.L. Stanford, Robust generation and expansion of skeletal muscle progenitors and myocytes from human pluripotent stem cells, Methods 101 (2016) 73–84.
[306] J. Chal, M. Oginuma, Z. Al Tanoury, B. Gobert, O. Sumara, A. Hick, F. Bousson, Y. Zidouni, C. Mursch, P. Moncuquet, O. Tassy, S. Vincent, A. Miyanari, A. Bera, J.-M. Garnier, G. Guevara, M. Hestin, L. Kennedy, S. Hayashi, B. Drayton, T. Cherrier, B.

Gayraud-Morel, E. Gussoni, F. Relaix, S. Tajbakhsh, O. Pourquié, Differentiation of pluripotent stem cells to muscle fiber to model Duchenne muscular dystrophy, Nat. Biotechnol. 33 (2015) 962.
[307] S. Iovino, A.M. Burkart, L. Warren, M.E. Patti, C.R. Kahn, Myotubes derived from human-induced pluripotent stem cells mirror in vivo insulin resistance, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) 1889–1894.
[308] R. Visone, M. Gilardi, A. Marsano, M. Rasponi, S. Bersini, M. Moretti, Cardiac meets skeletal: what's new in microfluidic models for muscle tissue engineering, Molecules 21 (2016).
[309] G.E. Salazar-Noratto, F.P. Barry, R.E. Guldberg, Application of biomaterials to in vitro pluripotent stem cell disease modeling of the skeletal system, J. Mater. Chem. B 4 (2016) 3482–3489.
[310] G. Cittadella Vigodarzere, S. Mantero, Skeletal muscle tissue engineering: strategies for volumetric constructs, Front. Physiol. 5 (2014) 362.
[311] S. Levenberg, J. Rouwkema, M. Macdonald, E.S. Garfein, D.S. Kohane, D.C. Darland, R. Marini, C.A. van Blitterswijk, R.C. Mulligan, P.A. D'Amore, R. Langer, Engineering vascularized skeletal muscle tissue, Nat. Biotechnol. 23 (2005) 879.
[312] L. Madden, M. Juhas, W.E. Kraus, G.A. Truskey, N. Bursac, Bioengineered human myobundles mimic clinical responses of skeletal muscle to drugs, Elife 4 (2015), e04885.
[313] G. Agrawal, A. Aung, S. Varghese, Skeletal muscle-on-a-chip: an in vitro model to evaluate tissue formation and injury, Lab Chip 17 (2017) 3447–3461.
[314] K. Shimizu, H. Araki, K. Sakata, W. Tonomura, M. Hashida, S. Konishi, Microfluidic devices for construction of contractile skeletal muscle microtissues, J. Biosci. Bioeng. 119 (2015) 212–216.
[315] C.G. Anene-Nzelu, K.Y. Peh, A. Fraiszudeen, Y.H. Kuan, S.H. Ng, Y.C. Toh, H.L. Leo, H. Yu, Scalable alignment of three-dimensional cellular constructs in a microfluidic chip, Lab Chip 13 (2013) 4124–4133.
[316] D.T.T. Nguyen, D. van Noort, I.-K. Jeong, S. Park, Endocrine system on chip for a diabetes treatment model, Biofabrication 9 (2017), 015021.
[317] Y.C. Toh, C. Zhang, J. Zhang, Y.M. Khong, S. Chang, V.D. Samper, D. van Noort, D.W. Hutmacher, H. Yu, A novel 3D mammalian cell perfusion-culture system in microfluidic channels, Lab Chip 7 (2007) 302–309.
[318] S. Bauer, C. Wennberg Huldt, K.P. Kanebratt, I. Durieux, D. Gunne, S. Andersson, L. Ewart, W.G. Haynes, I. Maschmeyer, A. Winter, C. Ammala, U. Marx, T.B. Andersson, Functional coupling of human pancreatic islets and liver spheroids on-a-chip: towards a novel human ex vivo type 2 diabetes model, Sci. Rep. 7 (2017), 14620.
[319] E. Iori, B. Vinci, E. Murphy, M.C. Marescotti, A. Avogaro, A. Ahluwalia, Glucose and fatty acid metabolism in a 3 tissue in-vitro model challenged with normo- and hyperglycaemia, PLoS One 7 (2012), e34704.
[320] L. Gnudi, R.J. Coward, D.A. Long, Diabetic nephropathy: perspective on novel molecular mechanisms, Trends Endocrinol. Metab. 27 (2016) 820–830.
[321] L. Wang, T. Tao, W. Su, H. Yu, Y. Yu, J. Qin, A disease model of diabetic nephropathy in a glomerulus-on-a-chip microdevice, Lab Chip 17 (2017) 1749–1760.
[322] E.M. Vedula, J.L. Alonso, M.A. Arnaout, J.L. Charest, A microfluidic renal proximal tubule with active reabsorptive function, PLoS One 12 (2017), e0184330.
[323] G. Ligresti, R.J. Nagao, J. Xue, Y.J. Choi, J. Xu, S. Ren, T. Aburatani, S.K. Anderson, J.W. MacDonald, T.K. Bammler, S.M. Schwartz, K.A. Muczynski, J.S. Duffield, J. Himmelfarb, Y. Zheng, A novel three-dimensional human peritubular microvascular system, J. Am. Soc. Nephrol. 27 (2016) 2370–2381.
[324] K.J. Jang, K.Y. Suh, A multi-layer microfluidic device for efficient culture and analysis of renal tubular cells, Lab Chip 10 (2010) 36–42.
[325] K.J. Jang, A.P. Mehr, G.A. Hamilton, L.A. McPartlin, S. Chung, K.Y. Suh, D.E. Ingber, Human kidney proximal tubule-on-a-chip for drug transport and nephrotoxicity assessment, Integr Biol (Camb) 5 (2013) 1119–1129.
[326] J. Valdes, L. Trachsel-Moncho, A. Sahaboglu, D. Trifunovic, M. Miranda, M. Ueffing, F. Paquet-Durand, O. Schmachtenberg, Organotypic retinal explant cultures as in vitro alternative for diabetic retinopathy studies, ALTEX 33 (2016) 459–464.
[327] R.A. Kowluru, Diabetic retinopathy, metabolic memory and epigenetic modifications, Vis. Res. 139 (2017) 30–38.
[328] D.H. Jo, J.H. Kim, J.H. Kim, A platform of integrative studies from in vitro to in vivo experiments: Towards drug development for ischemic retinopathy, Biomed. Pharmacother. 69 (2015) 367–373.
[329] J. Pauty, R. Usuba, H. Takahashi, J. Suehiro, K. Fujisawa, K. Yano, T. Nishizawa, Y.T. Matsunaga, A vascular permeability assay using an in vitro human microvessel model mimicking the inflammatory condition, Nano 1 (2017) 103–113.
[330] S. Kim, H. Lee, M. Chung, N.L. Jeon, Engineering of functional, perfusable 3D microvascular networks on a chip, Lab Chip 13 (2013) 1489–1500.
[331] M.L. Moya, Y.H. Hsu, A.P. Lee, C.C. Hughes, S.C. George, In vitro perfused human capillary networks, Tissue Eng Part C Methods 19 (2013) 730–737.
[332] C.H. Mandavia, A.R. Aroor, V.G. Demarco, J.R. Sowers, Molecular and metabolic mechanisms of cardiac dysfunction in diabetes, Life Sci. 92 (2013) 601–608.
[333] F.M. Drawnel, S. Boccardo, M. Prummer, F. Delobel, A. Graff, M. Weber, R. Gerard, L. Badi, T. Kam-Thong, L. Bu, X. Jiang, J.C. Hoflack, A. Kiialainen, E. Jeworutzki, N. Aoyama, C. Carlson, M. Burcin, G. Gromo, M. Boehringer, H. Stahlberg, B.J. Hall, M.C. Magnone, K. Kolaja, K.R. Chien, J. Bailly, R. Iacone, Disease modeling and phenotypic drug screening for diabetic cardiomyopathy using human induced pluripotent stem cells, Cell Rep. 9 (2014) 810–821.
[334] M. Radisic, H. Park, S. Gerecht, C. Cannizzaro, R. Langer, G. Vunjak-Novakovic, Biomimetic approach to cardiac tissue engineering, Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 362 (2007) 1357–1368.
[335] H. Song, P.W. Zandstra, M. Radisic, Engineered heart tissue model of diabetic myocardium, Tissue Eng Part A 17 (2011) 1869–1878.
[336] A. Mathur, Z. Ma, P. Loskill, S. Jeeawoody, K.E. Healy, In vitro cardiac tissue models: current status and future prospects, Adv. Drug Deliv. Rev. 96 (2016) 203–213.
[337] G. Conant, B.F.L. Lai, R.X.Z. Lu, A. Korolj, E.Y. Wang, M. Radisic, High-content assessment of cardiac function using heart-on-a-chip devices as drug screening model, Stem Cell Rev. Rep. 13 (2017) 335–346.
[338] A. Mathur, P. Loskill, K. Shao, N. Huebsch, S. Hong, S.G. Marcus, N. Marks, M. Mandegar, B.R. Conklin, L.P. Lee, K.E. Healy, Human iPSC-based cardiac microphysiological system for drug screening applications, Sci. Rep. 5 (2015) 8883.
[339] V.Y. Sidorov, P.C. Samson, T.N. Sidorova, J.M. Davidson, C.C. Lim, J.P. Wikswo, I-Wire Heart-on-a-Chip I: three-dimensional cardiac tissue constructs for physiology and pharmacology, Acta Biomater. 48 (2017) 68–78.
[340] J.U. Lind, T.A. Busbee, A.D. Valentine, F.S. Pasqualini, H. Yuan, M. Yadid, S.-J. Park, A. Kotikian, A.P. Nesmith, P.H. Campbell, J.J. Vlassak, J.A. Lewis, K.K. Parker, Instrumented cardiac microphysiological devices via multimaterial three-dimensional printing, Nat. Mater. 16 (2016) 303.
[341] S. Ehnert, T. Freude, C. Ihle, L. Mayer, B. Braun, J. Graeser, I. Flesch, U. Stockle, A.K. Nussler, S. Pscherer, Factors circulating in the blood of type 2 diabetes mellitus patients affect osteoblast maturation - description of a novel in vitro model, Exp. Cell Res. 332 (2015) 247–258.
[342] J.B. Buse, S. Caprio, W.T. Cefalu, A. Ceriello, S. Del Prato, S.E. Inzucchi, S. McLaughlin, G.L. Phillips, R.P. Robertson, F. Rubino, R. Kahn, M.S. Kirkman, How do we define cure of diabetes? Diabetes Care 32 (2009) 2133–2135.
[343] R.R. Rubin, M. Peyrot, Quality of life and diabetes, Diabetes Metab. Res. Rev. 15 (1999) 205–218.
[344] J.L. Chiang, M.S. Kirkman, L.M.B. Laffel, A.L. Peters, Type 1 diabetes through the life span: a position Statement of the American Diabetes Association, Diabetes Care 37 (2014) 2034–2054.
[345] D. Bertsimas, N. Kallus, A.M. Weinstein, Y.D. Zhuo, Personalized diabetes management using electronic medical records, Diabetes Care 40 (2017) 210–217.
[346] F. Lin, E.C. Butcher, T cell chemotaxis in a simple microfluidic device, Lab Chip 6 (2006) 1462–1469.
[347] I. Halilovic, J. Wu, M. Alexander, F. Lin, Neutrophil migration under spatially-varying chemoattractant gradient profiles, Biomed. Microdevices 17 (2015) 57.
[348] S. Han, J.-J. Yan, Y. Shin, J.J. Jeon, J. Won, H.E. Jeong, R.D. Kamm, Y.-J. Kim, S. Chung, A versatile assay for monitoring in vivo-like transendothelial migration of neutrophils, Lab Chip 12 (2012) 3861–3865.