Organoids from adult liver and pancreas: stem cell biology and biomedical utility

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ABSTRACT

The liver and pancreas are critical organs maintaining whole body metabolism. Historically, the expansion of adult-derived cells from these organs *in vitro* has proven challenging and this in turn has hampered studies of liver and pancreas stem cell biology, as well as being a roadblock to disease modelling and cell replacement therapies for pathologies in these organs. Recently, defined culture conditions have been described which allow the *in vitro* culture and manipulation of adult-derived liver and pancreatic material. Here we review these systems and assess their physiological relevance, as well as their potential utility in biomedicine.

KEYWORDS: organoid, 3D cultures, pancreas, liver, niche signals, stem cell

1. INTRODUCTION

The liver and pancreas are solid tissues which arise from the endoderm during vertebrate development (Figure 1). Amongst other functions, the liver and pancreas collectively control whole organism metabolism, with the pancreas primarily being responsible for the control of glucose homeostasis and the liver being responsible for detoxification and the production of urea. Both organs also secrete enzymes (pancreas) or solubilising factors (bile from the liver) into the intestine to aid in digestion, through the common hepatopancreatic duct, formed by the joining of the common bile duct and the pancreatic duct (Figure 1). In addition to similarities in function and morphology in adulthood, the liver and the pancreas share a common developmental history, set of early morphological patterning events and early transcription factors [1]. Despite the multiple shared characteristics of these organs, there are notable differences, key amongst them being that the adult pancreas is generally considered to have a much lower regenerative capacity than the liver [2]. Given their critical importance in organism physiology, it is not surprising that damage to either tissue can result in severely debilitating diseases. Although the liver is well known for its remarkable regenerative capacity, repeated damage to the tissue can result in cirrhosis and impairment of liver function [3]. In the pancreas, the ablation or loss of function of insulin-secreting β cells leads to diabetes mellitus and an inability to control glucose levels. For both organs, inflammation-related diseases such as hepatitis (liver) or pancreatitis (pancreas) leads to an increased risk of cancer, with the global incidence of both hepatic and pancreatic cancer increasing rapidly [4, 5]. Stem cell biology and regeneration in the adult liver and pancreas is therefore a field of interest for both the basic and biomedical communities. However, it has been difficult to explore this using *in vitro* methods, as primary cultures of both tissues display little expansion potential *in vitro*.

Recently, we and others have turned to 3D cell culture systems in order to investigate the stem cell biology of the liver and pancreas. The structures generated in such culture systems have been termed “organoids”. Here we adhere to our previous definition of organoid [6] as a 3D structure derived from either pluripotent stem cells (PSCs), neonatal tissue stem cells or adult-derived stem/progenitor cells (AdSCs), in which cells spontaneously self-organize into structures that resemble the in vivo tissue in terms of cellular composition and tissue function. When derived from AdSCs, these share the characteristics of *bona fide* stem cells as described by others [7], namely: 1) self-renewal of a stem cell population, usually for prolonged periods, 2) clonal expansion capacity, and 3) multipotency, with the stem cell population able to generate at least some of the other cell types seen in the adult tissue *in vivo.* In this review, we will be discussing the origins and characteristics of AdSC-derived liver and pancreas organoid cultures as currently described. We will consider each organ separately in term of its *in vivo* biology and the role that 3D *in vitro* culture has played in uncovering mechanisms of AdSC

biology. We will also discuss the role that studies of developmental biology have played in investigations of AdSC biology and the physiological relevance of such AdSC culture systems. Finally, we will summarise the biomedical utility of adult liver and pancreas organoid cultures in the fields of regenerative medicine and disease modelling.

2. LIVER ORGANOID CULTURES

Stem cells during liver development

During embryogenesis, the anterior-posterior patterning of naïve endoderm gives rise to an epithelium with foregut, midgut and hindgut identities, amongst which the ventral foregut contains common precursors for the liver and the pancreas (Figure 1) [1]. This progenitor pool is shared for both organs until embryonic day E8.5 in the mouse, when the segregation of presumptive pancreas and liver occurs [8]. The lineage choice of these precursors relies on a concentration gradient of mesodermal-derived FGF and BMP signals [9-11], whereby higher concentrations specify hepatic fate at the expense of a pancreatic one and *vice versa* [12, 13]. Committed hepatic progenitors - collectively referred to as hepatoblasts- are bipotential cells that give rise to the two main epithelial cell types of the liver: hepatocytes and cholangiocytes [14] and whose journey through development is carefully guided by cues from the microenvironment. The budding of the liver as an organ begins at E9.5 in the mouse with the migration of hepatoblasts into the adjacent septum transversum mesenchyme, a process that also requires the presence of endothelial cells despite the absence of blood flow [15] (Figure 1). At later stages, immature 'mesothelial' cells surrounding the liver parenchyma secrete mitogenic factors such as Hgf, Midkine, and Pleiotrophin [16] that induce hepatoblast expansion, whereas mature Thy1+ mesenchymal cells drive hepatoblast maturation by direct cell-to-cell contact as shown in a co-culture system [17]. During foetal development, the liver serves transiently as a site of hematopoiesis, a period in which it receives cytokines from blood cells [18, 19].

Hepatoblasts are a population of biomedical interest due to their bipotency, expansion potential and ability to contribute to adult liver regeneration when delivered to the site of damage. The collective knowledge from hepatogenesis has been harnessed to directly produce hepatocytes [20] or cholangiocytes [21] *in vitro* from pluripotent stem cell cultures (ESCs or iPSCs), by means of stepwise addition of defined soluble factors that the microenvironment normally provides *in vivo*. As an alternative to the provision of defined cues, Takebe and colleagues have demonstrated a strategy that incorporates embryonic mesenchymal and endothelial cells (MSCs and HUVECs) into a hiPSC-derived hepatoblast culture, resulting in the formation of self-organised 3D liver bud-like structures [22]. The discussion of such protocols is beyond the scope of this review and will be discussed in detail elsewhere in this issue, but the organoids and tissues formed by such methods are useful as a comparison to adult-derived organoid cultures. Interestingly, despite using populations that closely resemble *in vivo* hepatoblasts, currently described protocols do not allow the parallel generation of both hepatocytes and cholangiocytes in the same structure. As the regeneration of both hepatocyte and cholangiocyte compartments is a mainstay of adult liver biology, such bipotency is a key quality in assessing the stemness and biomedical utility of organoids generated *in vitro*.

Adult liver stem cells and stromal signalling

While hepatoblasts behave as the functional stem cell of the liver during development, they are generally not believed to persist postnatally; the healthy adult liver is a slow cycling tissue where epithelial maintenance occurs through the division of diploid cells [23, 24], the self-duplication of

mature cells [25, 26], and, albeit minimally, through a dedicated AdSC population [14]. Whilst largely quiescent during homeostasis, the liver is nonetheless capable of remarkable regeneration when challenged (Figure 2). Surgical removal of up to 70% of liver mass - referred to as partial hepatectomy- leads to compensatory proliferation and hyperplasia of hepatocytes, restoring tissue mass after just one week (reviewed by Michalopolous and DeFrances [27]). Yet, even when hepatocyte proliferation is compromised, for example during certain forms of toxic injury, the liver is still capable of repairing itself. This property of the liver has given rise to the theory of a facultative AdSC population, which has largely polarised the field in terms of its existence [28, 29].

Pioneering work dating back to the 1950s first described the appearance of small, proliferative, oval-shaped cells in adult rat livers with early stage carcinomas induced by ethionine or 2-acetylaminofluorene [30]. These cells emerge in the vicinity of biliary ducts and contribute to the regeneration of both cholangiocytes and hepatocytes (Figure 2) [31]. Morphologically comparable adult liver progenitors have been detected in mice using alternative hepatotoxic regimens such as the choline-deficient ethionine-supplemented (CDE) diet and administration of the carcinogens 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) or carbon tetrachloride (CCl_4) [32-34]. The cellular origin of these facultative progenitors has been widely believed to be a subpopulation of the ductal/biliary compartment of the liver. Destruction of the biliary tree by 4,4'-methylenedianiline (MDA) in rats impairs the proliferation of progenitors in models of oval cell activation [35]. Further to this, the existence of bipotent, facultative progenitors of a ductal origin is supported by lineage tracing *in vivo* in damaged livers from cells expressing markers such as *Sox9*, *FoxL1* and *Lgr5* [36-39], together with *in vitro* characterisation of these cells. However, a biliary origin for the oval cell has recently been challenged by data from the Willenbring and Stanger labs using viral-mediated Cre lineage tracing, which convincingly demonstrates that the repair of the damaged liver relies predominantly on the hepatocyte compartment [26, 40]. One concept which is able to reconcile these data is that of cellular plasticity, whereby the epithelial cell compartments of the liver could be capable of interconversion upon damage and thus able to repair one another [41, 42]. Indeed, recent studies have shown that the downregulation of Hippo signalling is sufficient to convert hepatocytes into cells expressing classical markers of biliary cells (e.g. panCK, Sox9) and oval cells (e.g. MIC1-1C3), although as these studies were performed using overexpression of an active form of YAP it is not entirely clear as to how frequently such interconversion events may occur under physiological conditions [43]. The concept of cellular plasticity has not been applied exclusively to the hepatic epithelium, yet it is possible that the evolutionary pressure applied by continuous metabolism of toxic waste has made it a more frequent and obvious necessity in this organ (Figure 2). This subject is comprehensively reviewed elsewhere [44, 45].

Adult liver architecture can be functionally subdivided into hexagon-shaped 'lobules' containing a central vein in the middle and 'portal triads' of arteries, veins and bile ducts at the periphery. Extending throughout the lobule there are also liver sinusoidal endothelial cells (LSECs) lining the tissue microvasculature, liver resident macrophages (Kupffer cells), lymphoid cells, hepatic stellate cells (HSCs) and myofibroblasts (Figure 2). Whilst the origin of the regenerative cell type in the liver continues to be contentious, a natural leap has been to focus instead on the signals that control regeneration. Similar to the role of stromal-derived cues in the embryo, stromal-derived cues in the adult liver could regulate tissue production following damage. Such an idea is an extension of the concept of AdSC 'niches': specialised microenvironments capable of influencing stem cell fate decisions like self-renewal and differentiation [46]. Although this concept was originally developed in the field of adult hematopoietic stem cells, it has now been expanded to epithelial tissues, such as the small intestine [47]. Following surgical resection of the liver, sinusoidal endothelial cells support tissue regeneration by secreting HGF and Wnt2, which increase hepatocyte proliferation [48]. Likewise, endothelial cells of the central vein appear to secrete Wnt9b and Rspo3 in addition to Wnt2 during

homeostasis [23, 49], although it is not known whether these are secreted following damage. IL22 secretion, which contributes to both hepatectomy-induced regeneration and to the inflammatory response during chronic hepatitis B virus infection that leads to progenitor proliferation [50], has been recently attributed to innate lymphoid cells of the liver [51]. Of interest, this molecular mechanism also seems to be conserved during injury in the small intestine [52]. Takase and colleagues showed that Thy1+ mesenchymal cells (including HSCs and myofibroblasts) induce the activation and proliferation of murine liver progenitors through Fgf7 secretion, such that forced overexpression of this factor alone is sufficient to stimulate progenitor emergence in healthy livers [53] (Figure 3). In chronic models of liver damage, Jagged1 expression at the surface of myofibroblasts has been shown to favour differentiation of progenitors towards the ductal lineage via Notch activation, whereas macrophage-derived Wnt3a has been associated with increased hepatocyte commitment [54]. The latter observation must however be reconciled with the reported role of Wnt in driving progenitor proliferation [23, 39]. One study found the cytokine profile of HSCs to change from pro- to anti-proliferative (HGF to TGFb) at different time-points following CCl_4 administration [55], thus highlighting the dynamic properties of the niche. It will thus be important to address the step-wise involvement of niche-derived signals in driving progenitor dynamics at each stage of regeneration *in vivo*, similar to work performed during embryonic liver development.

Adult-derived organoids from the liver

The culture of liver AdSCs has historically proven to be challenging, in spite of the liver's remarkable capacity to proliferate following damage, but studies of liver regeneration *in vivo* have provided the foundations for defined culture conditions that promote the expansion of liver AdSCs *in vitro* (Figure 3). We have shown that the Wnt target gene leucine-rich-repeat-containing G-protein-coupled receptor 5 (*Lgr5)* labels actively proliferating cells in the adult mouse liver following toxic damage; these cells are capable of differentiating into both hepatocytes and cholangiocytes *in vivo,* and thus meet the criteria of a bi-potential AdSC population [39]. Single $Lgr5^+$ cells can be cultured in Matrigel™, an extracellular matrix containing collagen and laminin, and under growth factor-defined conditions that include Hgf, Fgf, Egf and, crucially, the Wnt agonist Rspondin1, which is the ligand for Lgr5 [56]. $Lgr5^+$ cells expand and self-organise into 3D cystic structures comprised of a single-layer epithelium expressing a mixture of progenitor (*Lgr5, Trop2*), ductal (*Krt7, Krt19*) and hepatocyte (*Ttr, Hnf4a*) markers. Interestingly, placing healthy biliary ducts under the same culture conditions leads to the formation of phenotypically identical organoids [39]. Employing a similar strategy, we further established human hepatic organoids from both healthy liver biopsies and single $EpCAM^+$ cells [57]. This required modification of the previous media conditions to include inhibition of the TGF-β signalling pathway and activation of cAMP signalling (Figure 3). Human adult liver-derived organoids express the progenitor marker *LGR5* and display a mixture of hepatocyte and ductal markers, thus exhibiting phenotypic similarity to murine adult liver-derived organoids. Such similarities suggest a conservation of progenitor biology across species in the liver, although specific signalling pathways promoting the progenitor state may vary.

Support for a conserved progenitor population and response comes from the derivation of adult 3D liver organoids from other mammalian model systems, for example canines [58]. Whilst dog-derived liver organoid cultures recapitulate the features of their mouse and human counterparts, they are unique in that their continuous self-renewal relies on Bmp inhibition and Wnt activation achieved by supplementing the medium with Noggin and Wnt3a, respectively; signals which are otherwise dispensable in the former systems. In conjunction with the human, canine liver organoids do not maintain proliferation potential past 3 months without inhibition of Tgf-β signalling (Figure 3). Kuijk and colleagues have most recently added to the list of species from which adult liver organoids can be generated by deriving rat liver organoid cultures [59]. Rats are a particularly interesting model for

liver regeneration because of their well-defined progenitor response following injury [47, 60], yet somewhat paradoxically, previous efforts to expand these progenitors have been unsuccessful [61]. Rat organoids proliferate extensively *in vitro* and resemble canine ones in their dependence on addition of exogenous Wnt and Noggin to maintain the culture, although similar to the mouse they do not require Tgf-β inhibition [59]. On the contrary, rat liver organoids cannot maintain proliferation past a week when cultured in human organoid medium, suggesting that either Tgf-β inhibition or cAMP activation is detrimental for the self-renewal of rat liver progenitors (Figure 3). Whether differences in Tgfb-signalling could explain species-specific abilities to activate tissue progenitors *in vivo* is an interesting question which is yet to be addressed.

A remarkable feature of all adult-derived liver organoid cultures to date, especially considering the historical difficulties of primary liver cell culture, is their prolonged capacity for self-renewal (up to 1 year) at a population doubling rate of 48-60h [39, 57]. Despite this high number of cell divisions, organoid cells do not show signs of transformation: studies in human organoids have shown remarkable genomic stability at the chromosomal and nucleotide level even at late passages [57]. This is an important difference to iPSC-derived organoid cultures, which are often found to have accumulated genetic aberrations, most likely caused by the process of reprogramming [62]. All organoid cultures studied so far retain the ability to differentiate into functionally mature hepatocytes upon withdrawal of proliferative stimuli and the modulation of key signalling pathways that also appear to vary slightly according to the species. For instance, addition of dexamethasone in combination with inhibition of Notch and TGF-β signalling is sufficient to drive murine organoid differentiation, while BMP7 is required as an additional component when directing the differentiation of human liver organoids to hepatocytes. The lack of spontaneous hepatocyte differentiation in the liver organoids contrasts greatly with that of their intestinal counterparts, where all differentiated cell types of the crypt-villus axis are produced *in vitro* from a single $Lgr5^+$ cell [63]. Although it is tempting to attribute this to the innate difference in tissue homeostasis between both organs *in vivo* (damage-induced facultative progenitors vs rapidly cycling, bona-fide stem cells 'pre-programmed' to replace all cell types), organoids from other rapidly cycling epithelial tissues, such as the stomach, also require external signalling inputs to drive differentiation into mature cell types [64].

3. PANCREATIC ORGANOID CULTURES

Stem cells in pancreatic development and in the adult

During embryonic development, unlike the liver, the pancreas undergoes simultaneous development in both the ventral and dorsal anterior foregut, with the dorsal pancreatic epithelium budding into its surrounding mesenchyme and developing separately from the ventral pancreatic bud before morphogenetic movements cause both tissues to merge around E13 in the mouse and 7-8 weeks post-conception in the human [65]. The multipotent progenitors of the embryonic pancreas will form epithelial ducts terminating in acinar cells interspersed with endocrine cells segregated into the islets of Langerhans. To form the islets, ductal trunk progenitors will delaminate from the developing ductal tree and migrate to form polyclonal endocrine clusters, which will develop into the mature islets [66, 67]. The adult pancreas is thus composed of three functional compartments arising from separate morphogenetic events in development: acinar cells derived from tip cells, ducts remodelled from an epithelial plexus and endocrine islets derived from migrating trunk cells (Figure 4).

The first lineage segregation events occur early in pancreas development when the pancreatic bud undergoes separation into tip and trunk cells, with tip cells being identified through expression of Cpa1 [68]. As the epithelium develops, tip cells will differentiate into acinar cells whilst trunk cells will

differentiate into the duct and endocrine lineages. The expression of the basic helix-loop-helix transcription factor Neurogenin3 (Neurog3) would appear to be a critical determinant of endocrine fate during pancreatic development, as trunk cells expressing Neurog3 will delaminate from the forming ductal tree and migrate to form the islets [69, 70]. The islets themselves would appear to mature and expand predominantly during postnatal stages, by which time the lineages within the pancreas are highly segregated. Indeed, homeostatic maintenance of the adult pancreas would seem to be highly compartmentalised, with the majority of reports demonstrating self-duplication of acinar and ductal compartments [71-73], with further compartmentalisation within the islets demonstrated by self-duplication of β cells [74, 75]. Under homeostatic conditions, interconversion between cell types in the healthy adult pancreas would appear to be very low, if not completely absent, and such segregated homeostatic turnover is highly comparable to homeostasis in the adult liver (cf Figures 2 and 4). This compartmentalisation has also been reported to be maintained during damage and regeneration, with the specific ablation of β cells resulting in an increased rate of replication in remaining β cells [75, 76]. It is tempting to speculate that the strict compartmentalisation observed in the pancreas leads to its lower regenerative capacity when compared to the liver. However, compartmentalisation of proliferative niches is also well documented in the skin, and yet this organ has full regenerative capacity through transient cellular plasticity, which allows for stem cells from one compartment to contribute to the repair of other compartments during damage [77]. It is also worth placing the regenerative capacity of the pancreas into context, as the organ is capable of full functional recovery following certain types of damage, such as partial duct ligation [78], and estimates suggest that as much as 90% of β cell mass must be lost before clinical signs of diabetes become apparent [79]. It is certainly true though that the pancreas is not capable of the rapid regeneration exhibited by the liver to a wide range of physical and toxic insults.

The comparatively lower regenerative capacity of the pancreas, coupled with the rise in incidence of both type1 and type2 diabetes, has led to a search for alternative regenerative mechanisms or cell sources which could produce new, functional β cells. In contrast to the liver, there is no morphologically distinct cell population which arises during pancreatic regeneration, which has made the existence of a facultative, multipotent AdSC population in the pancreas an even more contentious issue than for the liver [44]. It would appear that under certain forms of damage, interconversion between cell types is possible but highly inefficient. Studies into plasticity in the damaged pancreas have been largely informed by events during pancreatic development, particularly the close association between the ductal and endocrine lineages that has led many groups to investigate whether the mature ductal tree has any capacity for endocrine cell production upon damage in the adult. Several groups have reported the reactivation of Neurog3 expression in damaged ducts, followed by the formation of novel islets in close association with the ducts [80-83]. The expression of Neurog3 in ducts would suggest a dedifferentiation event or the reacquisition of developmental potential. However, it is currently unclear as to how mature the newly formed endocrine cells are and further, in spite of the acquisition of Neurog3 expression in some ductal cells, which is the cell of origin for these new endocrine clusters (Figure 4). Studies in zebrafish have demonstrated that centroacinar cells, ductal cells at the acinar termini with elongated cytoplasmic projections, are a cell of origin for the production of new endocrine cells [84]. However, it is not clear that mammalian centroacinar cells have the same potential to form endocrine cells during damage and the vastly increased capacity of the zebrafish pancreas to regenerate would suggest that mechanisms operating in the zebrafish may not be present in mammals. Rather than rely on endogenous mechanisms of generating new islets, other groups have attempted to direct the transdifferentiation of alternative cell types to β cells. It is clear that the overexpression of specific transcription factors can result in the interconversion of α cells, duct cells or even acinar cells to β cells *in vivo* [82, 85, 86]. However, whilst

informative in terms of the underlying molecular circuitry of tissue compartmentalisation and pancreatic plasticity, it is questionable as to whether such approaches will ultimately be of biomedical utility.

Embryonic organoids from the pancreas

Given the paucity of β cell neogenesis in the adult pancreas, studies of the developing pancreas have provided most of our knowledge on the molecular circuitry of the cell types in the adult pancreas. The development of the mammalian pancreas can be studied *ex vivo* by culture with a basic medium at the air/liquid interface, a culture system first described in 1954 [87]. The use of this system not only revealed the instructive role of the mesenchyme during pancreatic development [88], but further suggested that a defined set of signals would be capable of directing a population of multipotent progenitors present in the embryonic pancreas. Whilst *ex vivo* culture allowed the direct investigation of pancreatic development by addition of soluble factors to direct differentiation of pancreatic explants, the expansion potential of the explants was limited and further manipulation of the explants at the interface was difficult. In particular, *ex vivo* explants could not address the question of whether multipotent progenitors required cell-cell interactions for their maintenance and/or differentiation, as only entire explants rather than single cells can be cultured using this method. However, by defining factors involved in the development of the pancreas, such explant cultures have led to the more recent description of culture conditions in which single cells from the E10.5 mouse pancreas can be maintained. The embryonic pancreas progenitors grow into 3D organoids which can be expanded for up to 2 weeks in culture and retain their potential to differentiate into acinar, ductal or endocrine lineages [89]. The embryonic pancreas organoids undergo rapid proliferation and can recapitulate the branching structure of the pancreatic epithelium. Because the cells maintain their ability to efficiently differentiate into all three lineages, the system is useful for studying signals which bias cell fate decisions towards particular lineages. For example, the authors demonstrated that FGF and Notch signalling were necessary but not sufficient for the formation of organoids and maintenance of progenitor status, mirroring the role of these pathways *in vivo* during development [90-92]. Also, the authors found a role for community effects, as organoids formed more efficiently and maintained their progenitor status for longer periods when cultured as groups of cells rather than single cells. Such analyses underline the importance of understanding niche signals at the population level in order to direct cell fate decisions.

Adult-derived organoid cultures from the pancreas

The demonstration of multipotency in organoids formed from embryonic progenitors is an important biomedical property, and an *a priori* requirement for a facultative AdSC capable of regeneration. The use of *in vitro* culture to demonstrate both expansion potential and multipotency is thus an important alternative route to identifying cells with the capacity to become a facultative AdSC, even if such properties are not present or active *in vivo*. Using CD133 as a marker, it has been possible to isolate cells from the adult mouse pancreas, which demonstrated expansion potential and multipotency *in vitro* in 3D culture [93, 94]. However, organoids grown under these conditions did not display long-term self-renewal potential and were heterogeneous in their morphology. By contrast, organoids formed from adult human $CD133^+$ pancreatic cells were ductal in phenotype and grew as homogeneous, hollow spheres composed of a single-layer epithelium [95]. However, endocrine differentiation potential was only reported following transfection of the organoids with vectors expressing transcription factors known to promote endocrine cell fate. As these factors have been reported to induce transdifferentiation of acinar cells to β cells *in vivo* [85], it is unclear as to whether these organoids are multipotent.

Recently we described the long-term culture of organoids derived from the adult mouse [96] and human [97] pancreas using a novel culture system. Following on from our observation that pancreas damage by means of pancreatic duct ligation results in upregulation of canonical Wnt signalling [96], we defined culture conditions which promoted the growth of organoids by the addition of Rspondin-1, the Lgr5 ligand, and co-activator of the pathway [56]. Thus, pancreas cells could be cultured and give rise to cystic, single-layer organoids, which express ductal markers such as Sox9 and keratin19 as well as canonical stem cell markers such as Lgr5 [44, 98] or CD133 [99]. Most strikingly, mouse pancreas organoids could be expanded in culture for at least 5 months whilst retaining expansion potential [96]. These organoid cultures can also act as bipotent progenitors, being capable of forming endocrine cells following grafting into the rat kidney capsule. In searching for the cell-of-origin for the organoids, Synaptophysin$^+$ (endocrine) cells were demonstrated to lack organoid-forming potential, and Ptf1a$^+$ (acinar) cells generated organoids, which could be maintained for only a few passages *in vitro*. By contrast, Sox9$^+$ ductal cells were shown to form organoids which could be maintained long-term, in a manner reminiscent of the ductal cell-of-origin for adult liver-derived organoids. Further similarity to adult liver-derived organoids was demonstrated by showing that healthy pancreatic ducts could give rise to long-term proliferative organoid cultures when placed into the defined culture conditions. The similarity of organoid cultures derived from Lgr5$^+$ cells arising in the liver and pancreas upon damage has been further highlighted by the report that organoids derived from the adult mouse pancreas can transdifferentiate to form hepatocytes in the damaged liver [100].

Although Lgr5 expression defines a bipotent population which maintains expansion potential *in vitro*, the ability of organoids to differentiate to an acinar, exocrine fate was not assessed and the Lgr5$^+$ population was not traced *in vivo* to demonstrate incorporation into regenerated endocrine or acinar compartments. In addition, the endocrine differentiation capacity of the organoids was very low and although detection of C-peptide suggests that β cells formed from organoids are mature, no functional tests of insulin release following glucose stimulation or transplantation into diabetic rodent models were undertaken. Thus, the ductal organoids generated may accurately mirror a true *in vivo* population in the pancreas, as a variable efficiency of ductal to endocrine conversion has been reported specifically following partial duct ligation [80, 101]. In addition, it is unclear as to the physiological role that Lgr5 expression plays in the adult pancreas during damage, given that our report of a proliferating progenitor conflicts with the use of Rspondin-1 by others to induce endocrine differentiation [93]. Identifying the crucial differences between culture systems that lead to differing cell responses will be a key step towards fully controlling cell fate decisions *in vitro*.

4. BIOMEDICAL UTILITY OF ADULT-DERIVED ORGANOID CULTURES

Discussion above has focussed on the establishment of organoid systems and whether they represent a physiologically accurate state. However, as we address below, we believe that organoid technology can go beyond this and could contribute to significant biomedical advances by modelling disease states (Figure 5).

Cell therapy

Several pathologies develop from the failure of the functional cells within the tissue (Figures 2 and 4). Considering the many roles of the liver in regulating whole-body homeostasis, it is not surprising that loss of function in the hepatocyte compartment leads to hepatic diseases which are often fatal [102]. Currently, orthotopic liver transplantation is the only effective treatment for end-stage hepatic failure, yet this method is completely reliant on organ donations that are both limited and

unpredictable [103]. In the pancreas, type 1 diabetes mellitus, results from the loss of β cell function. Islet transplantation has proved a successful method for curing diabetes, but the supply of islets is limited, even more so than with liver tissue as cadaveric islets must be used. In both cases, the recipient is most often required to undertake an immunosuppressive drug regimen for the rest of their life [104, 105], an outcome that might not be required if sufficient replacement tissue could be generated from the patient themselves. The generation of replacement cells or tissue *in vitro* is thus a cornerstone of regenerative medicine approaches for both organs, to circumvent inadequacies in the supply of donor tissue and the requirement for immunosuppression post-transplantation.

Given their combined expansion and differentiation potential, organoids could serve as a source of donor tissue expanded and manipulated *ex vivo* before re-introduction into the patient (Figure 5). This concept has been predominantly explored using liver organoids (also cf. engraftment of intestinal organoids into damaged mouse gut epithelium in [106]), with no reports of organoid-generated endocrine cells being used to alleviate symptoms in diabetic models to date. As a proof of principle for the concept of organoids as a source material for transplantation in the liver, differentiated mouse liver organoids were transplanted into $Fah^{-/-}$ mice, a model for tyrosinaemia type I liver disease. Organoid-derived $Fah^{+/+}$ hepatocytes occupied a modest 1% of the liver parenchyma, yet despite this managed to increase survival. Compared to 'gold-standard' fresh hepatocyte transplantations, wherein tissue replacement exceeds 30% and functional rescue is almost complete, organoid graft performance is considerably below par. On the other hand, $MIC1\text{-}1C3^{+}$ 'oval cells' have also displayed only trace engraftment [38], and it is likely that transplantation procedures could be optimised to increase effectiveness. Organoid graft success may depend on multiple factors, including the differentiation stage of cells introduced and the disease model. Takebe and colleagues have further shown that the mesenchymal and vascular components of their embryonic 'liver bud' organoids (and other iPSC-derived organ-buds, including pancreatic buds) are crucial for establishing communication with the host [22, 107]. The lack of stromal support in adult organoids could thus represent a disadvantage for therapeutic applications.

Human hepatic organoids have been tested in their ability to repair livers damaged with retrorsine/CCl_4, which causes central vein hepatocyte loss [108]. In this study, organoid cells were injected in their ductal progenitor state ($Krt19^{+}Alb^{-}$), yet switched to the hepatocyte lineage ($Alb^{+}Krt19^{-}$) *in vivo* and contributed to endogenous liver function, implying that host liver signals may drive progenitor fate choice [57]. Moreover, the specific type of liver damage (e.g. injuring the biliary vs the hepatocyte compartment) may pattern the microenvironment to direct transplanted cells towards one fate versus the other, as has been shown by Boulter and colleagues [54].

Disease modelling and gene therapy

At present, studies of adult liver function and dysfunction are strongly reliant on animal usage. Alternative *in vitro* models usually consist of primary hepatocyte cultures with limited lifespan potential and/or immortalised hepatic lines, such as HepG2 cells, that deviate from normal hepatic physiology [109]. In contrast, adult organoid cultures offer the advantages of genetic stability [57], active proliferation and bipotency, thus proving of great biomedical utility for modelling liver behaviour *in vitro* (Figure 5). We have shown that organoids derived from patients diagnosed with a1-antitrypsin deficiency (A1AT-D) recapitulate the features of the disease upon induction of hepatocyte differentiation [57]. The pathology of this disease is attributed to inherited mutations in the *SERPINA1* gene, which lead to misfolding and aggregation of the A1AT protein inside hepatocytes. This work would then suggest not only that mutant protein expression is preserved following organoid derivation but also that symptoms manifest themselves only under the appropriate cell context (i.e. in mature hepatocytes).

Modelling aspects of cancer biology *in vitro* would provide platforms for more accurate prognosis, prediction of disease progression and drug screening. In this regard, organoid technology provides several advantages, not least that the culture conditions allow the rapid expansion of primary material for downstream assays (Figure 5). As over 95% of pancreatic cancers arise from the exocrine compartment with mutation of KRAS seen in over 90% of these tumours [110], pancreatic ductal adenocarcinoma represents a relatively homogeneous pathology in modelling terms. Human adult-derived pancreatic organoids were seen to recapitulate the features of early- and late-stage cancer when derived from patients, including the increased expression of pancreatic adenocarcinoma markers (e.g CA19-9), chromosomal abnormalities and neoplastic morphology when xenografted in mice [97]. A similar study used the directed differentiation of pluripotent stem cells (PSCs) to the exocrine lineage to produce 3D organoid cultures which displayed tumorogenic characteristics following transfection with $KRAS^{G12D}$ or the dominant-negative mutant of p53, $TP53^{R175H}$ [111]. The authors then demonstrated that their PSC-derived organoid culture conditions were also suitable for growing primary tumour-derived organoids, using this platform to demonstrate the utility of organoid technology for personalised drug screening. Reciprocally, genes believed to have a functional role in cancer progression can be assessed in organoid cultures before extending such studies to the more expensive and time-consuming animal models. This concept was nicely demonstrated by Westphalen and colleagues when identifying the expression of Doublecortin-like kinase 1 (Dclk1) in a subpopulation of cells in the pancreas which are activated following damage induced by cerulein treatment or partial duct ligation [112]. The authors additionally demonstrated that $Kras^{G12D}$ provides resistance to EGF withdrawal in organoid culture only in the presence of functional Dclk1. This *in vitro* observation provided the foundation to uncover the role of $Dclk1^{+}$ cells as potent tumour-initiating cells in the presence of mutant Kras following pancreas damage.

The fact that 3D organoid cultures are amenable to genome-editing techniques (e.g. CRISPR/Cas9, lentiviral transduction), as best shown in pioneering work on gut organoids [113, 114], opens up numerous possibilities for disease and repair modelling *in vitro* (Figure 5). In the liver context, Nantasanti and colleagues have restored hepatic organoids from *COMMD1*-null dogs with the wild-type *COMMD1* gene and managed to restore their ability to excrete copper, a function that is otherwise impaired in the livers of these dogs and leads to copper-induced hepatitis [58]. Thus far, there are no reports utilising genome-editing in adult-derived pancreas organoid cultures, although we note that studies utilising gene correction of CFTR in intestinal organoids [113] would also be applicable to disease modelling in pancreas organoids, given the pancreatic phenotype seen in cystic fibrosis patients. Such an approach has been utilised in PSC-derived cholangiocytes in combination with drug screening to alleviate the effects of mutant CFTR [21]. While adult-derived hepatic organoids may prove useful tools for studying monogenic diseases of the epithelial liver compartment, liver disorders such as hepatocellular carcinoma and non-alcoholic fatty liver disease, pathologies where the cellular microenvironment has been identified as a key component of the disease [115, 116], may be more difficult to model accurately with these organoids. One way of circumventing this issue could be to establish co-cultures between adult liver organoids and stromal cells, an interaction which automatically occurs in parallel with the establishment of organoid cultures in PSC-derived systems [107, 117, 118].

5. CONCLUSION AND FUTURE PERSPECTIVES

Organoid cultures allow the expansion of adult liver and pancreatic material, which has not been possible using more traditional 2D culture methods. The establishment of these systems has allowed

investigation of the self-renewal and differentiation properties of these tissues *in vitro*. Similar to the use of *in vitro* hepatoblast and embryonic pancreas explant cultures for investigating the stem cell characteristics of these cells [14, 88], we can think of adult-derived organoids as *in vitro* platforms for modelling liver and pancreas regeneration in terms of progenitor activation, proliferation and differentiation. A caveat to this usage is that stem cell-like behaviour *in vitro* can be an artefact of removing cells from their native environment and placing them into conditions that promote and maintain non-physiological cell states [41]. However, we would point out that firstly, not all cells are capable of forming organoids *in vitro* (e.g. $EpCAM^-$ fraction), indicating lineage-specific competence in reverting to an uncommitted progenitor state. Secondly, the promotion or maintenance of states which are rare *in vivo* could be of great utility when searching for markers of the AdSC state. This is particularly relevant when considering primary pancreatic material, where the role of an *in vivo* AdSC state is particularly controversial [44]. Related to this, the defined nature of the culture conditions means that organoid cultures are ideal for studying stem cell-niche interactions in a 3D environment, which remains a relatively unexplored area for diseases such as hepatic and pancreatic cancer or type 2 diabetes.

In addition to their role in investigating stem cell biology, 3D organoid cultures promise to be of considerable biomedical utility. Given their rapid expansion potential, genomic stability and capacity to differentiate, adult-derived liver and pancreas organoids could serve as a cell source for assays requiring primary cells. In the short term, we foresee advances using the expansion potential of organoid culture to provide physiologically relevant assays at a high throughput level, both for normal and patient-derived organoids. Such assays could include use in personalised medicine approaches, as highlighted for colorectal cancer [119]. In the long term, we expect the advantages of adult-derived organoid technology as a genetically stable, non-tumourigenic source material capable of generating differentiated cell types [57, 58] to be combined with the advantages of a physiologically relevant 3D culture system in defining factors controlling cell fate decisions and result in significant advances in the field of cell therapy and regenerative medicine. Overall, we predict that organoids derived from the adult liver and pancreas will fulfil a crucial role in biomedicine by providing an accessible but physiologically relevant 3D *in vitro* model system.

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FIGURE LEGENDS

Figure 1. A shared hepatopancreatic ductal tree arises during embryogenesis. The liver and ventral pancreas arise from the same region of the ventral anterior foregut endoderm during early embryogenesis (left image), with both tissues budding into the surrounding mesenchyme later on in development (central image). The resulting organs in adulthood share a ductal network of epithelial cells (right image), with the secretory cells of each organ directly contacting the ductal network

(liver/hepatocytes, pink; pancreas/acinar cells, yellow). Lv: liver; Pc (d): dorsal pancreas; Pc (v): ventral pancreas; STM: septum transversum mesenchyme.

Figure 2. Tissue architecture of the healthy, regenerating and diseased liver. There is a low rate of proliferation in the healthy liver (upper panel) which is increased in all cell types upon damage and regeneration (middle panel). In addition to the appearance of an "oval cell" population during regeneration, the observed interconversion between cell types (arrows) suggests a high degree of tissue plasticity. In the diseased liver, chronically activated mesenchymal cells induce a large degree of tissue fibrosis along with impaired regenerative capacity and hepatocyte function. Under these conditions, tumours bearing characteristics of hepatocytes (HCC), cholangiocytes (CCA) or a mixture of characteristics are more likely to be formed.

Figure 3. Soluble growth factors *in vivo* and *in vitro*. Tables comparing the growth factors known to regulate liver regeneration *in vivo* and their cellular source with growth factors used for the culture of liver organoids *in vitro* and additional media components required *in vitro* are shown. There is a large degree of overlap between growth factors enhancing regeneration *in vivo* and those required for long-term expansion of organoids (upper table). Additional soluble factors required for long-term growth *in vitro* are highly dependent upon the species from which organoids are isolated (lower table).

Figure 4. Tissue architecture of the healthy and diseased pancreas. During normal homeostasis in the healthy pancreas, a low rate of proliferation in each compartment drives maintenance. Tissue plasticity is not seen under these conditions. In the disease state, immature endocrine cells can be found close to ducts in some models of diabetes and acinar cells undergo acinar-to-ductal metaplasia during pancreatic cancer progression. Although this tissue plasticity may suggest a facultative AdSC, the existence of such a cell is controversial and it is not shown in this figure.

Figure 5. Biomedical uses of adult organoid culture. (**A**) Organoids can be generated from healthy liver to provide an expandable source of hepatocytes for toxicological testing (lower images) or from diseased liver to allow *in vitro* disease modelling (upper images). Organoids from diseased livers can undergo gene correction and repopulate the diseased tissue as a form of cell therapy. (**B**) Organoids can be generated from healthy pancreas to provide an expandable source material for generating β cells for cell therapy (lower images) or from diseased pancreas to allow *in vitro* disease modelling and drug screening for personalised medicine approaches.

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