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REVIEW ARTICLE

# Engineering of microscale three-dimensional pancreatic islet models *in vitro* and their biomedical applications

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**Abstract**

Diabetes now is the most common chronic disease in the world inducing heavy burden for the people's health. Based on this, diabetes research such as islet function has become a hot topic in medical institutes of the world. Today, in medical institutes, the conventional experiment platform *in vitro* is monolayer cell culture. However, with the development of micro- and nano-technologies, several microengineering methods have been developed to fabricate three-dimensional (3D) islet models *in vitro* which can better mimic the islet of pancreases *in vivo*. These *in vitro* islet models have shown better cell function than monolayer cells, indicating their great potential as better experimental platforms to elucidate islet behaviors under both physiological and pathological conditions, such as the molecular mechanisms of diabetes and clinical islet transplantation. In this review, we present the state-of-the-art advances in the microengineering methods for fabricating microscale islet models *in vitro*. We hope this will help researchers to better understand the progress in the engineering 3D islet models and their biomedical applications such as drug screening and islet transplantation.

**Keywords**

Cell microenvironment, islet model, microengineering methods, three dimensional

**History**

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## Introduction

Diabetes is currently a major risk factor for cardiovascular diseases (CVD), where the incidence of type 1 diabetes mellitus (T1DM) is approximately 1 in 300 with a steady increase of 3% per year worldwide (Gan et al., 2012). In T1DM male patients, the hazard ratio for major CVD was 3.6 (95% CI 2.9–4.5) compared with those without diabetes and 7.7 (5.5–10.7) in females. Type 1 diabetic males aged 45–55 years have an absolute CVD risk similar to that of males in the general population who are ~10–15 years older, with an even greater difference in females (Soedamah-Muthu et al., 2006). For T1DM patients, insulin injection is currently the most common approach to maintain their lives. As an alternative, cell therapy through islet transplantation has emerged recently showing improved treatment effectiveness due to more mimic physiological insulin secretion compared with conventional subcutaneous insulin injection. However, lack of an islet cell source has limited the wide clinical applications of cell therapy, which requires a large number of islet cells (mostly at least two donor pancreases for one recipient) for transplantation (Robertson, 2004). In addition, immune rejections after islet transplantation as well as the decline of graft functions with time are other potential risks (Shapiro et al., 2006; Ricordi & Strom, 2004). Given the complexity of pancreas transplantation, only a small number of patients have taken this treatment and shown good therapeutic effectiveness in the end (Shapiro et al., 2000). Therefore, there is still an urgent need to develop an effective therapy for the diabetes. With the rapid development of biomedical engineering, one potential solution is to regenerate functional islets *in vitro* using a tissue engineering strategy (Soon-Shiong et al., 1994). Moreover, the engineered islet tissues as *in vitro* models also exhibit great potentials in broad applications such as high-throughput drug screening and islet cell biology study.

The microstructure of islets is complex and has shown to have a significant effect on the islet functionalities (e.g. β cell survival and insulin secretion). For instance, recent studies have shown that three-dimensional (3D) islet models (i.e. islet cell aggregations, also called pseudoislets) can stimulate more insulin secretion than monolayer cell culture (Bernard et al., 2012; Persaud et al., 2010). Intact islets of Langerhans have shown greater integrated secretory response than that of dispersed islet cells. Besdies, aggregations of the dispersed cells show improved secretory response, suggesting that

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intra-islet interactions are essential for secretory responses (Bosco et al., 1989; Halban et al., 1987; Hopcroft et al., 1985). Moreover, islet interaction such as connexin 36-dependent signaling is essential for proper functioning of β cells, particularly for the overactive ATP-insensitive $K_{ATP}$ channels and pulsatility of $[Ca^{2+}]_i$ which enhance insulin secretion (Nguyen et al., 2014; Ravier et al., 2005). Nowadays, *in vitro* diabetes research is usually conducted by culturing cells on two-dimensional (2D) flat, rigid plastic substrates that do not reproduce the native microarchitecture of 3D geometry, gel-like stiffness, and complex organization of ECM. In comparison, 3D cell cultures establish cell–cell and cell–ECM interactions that better mimic the biochemistry and mechanics of the native cell microenvironment. Therefore, it is important to recapitulate the 3D microarchitecture of native islets to regenerate the cell interaction and their functions *in vitro*, which could be utilized to study the physiology and the pathology of islets and eventually to be transplanted *in vivo* in order to treat diabetes.

The conventional methods for 3D islet models are to develop β cell aggregates via self-assembly either in static suspension or in non-adhesive tissue culture plates (O'Sullivan et al., 2010; Weber et al., 2006). Although this method is simple with no need of using special equipment, it has limited control over the composition ratio of different cells and the size of the fabricated islet tissues, resulting in huge size heterogeneity. Alternatively, with advances in the micro- and nanotechnologies, several microengineering methods have been developed to fabricate 3D islet models *in vitro* in a high-throughput manner. The hanging-drop method can re-aggregate islet cells into spherical clusters with a relatively uniform size (~100 μm in diameter) (Cavallari et al., 2007). Dielectrophoresis has been used to aggregate β cells into 3D constructs (Pethig et al., 2008). Microwell can also be used to fabricate 3D islet models with well-controlled sizes (Bernard et al., 2012). More recently, microcontact printing has been employed to fabricate β cell aggregates, which allows the control of aggregate sizes by changing the size of the printed area (Mendelsohn et al., 2010) (Table 1). Besides, all above methods using scaffolds with cell adhesive peptides is another most popular method to fabricate islet model. It can provide well-controlled 3D architecture and improved cell activity because of adequate nutrition and oxygen (De Carlo et al., 2010). However this method is not only the cell aggregate, so we do not compare it in Table 1.

Although there are numerous studies focusing on the methods of fabricating 3D islet structures *in vitro*, there are few reviews considering the comparison among these approaches. There exist several good reviews on the method of engineering tissues or organs *in vitro* (Atala et al., 2012; Eschenhagen & Zimmermann, 2005; Lutolf & Hubbell, 2005), however, fewer focus on pancreatic islet tissues. In this review, we present state-of-the-art advances in the microengineering methods for fabricating microscale islet model *in vitro*. We hope this will help researchers to better understand the progress in constructing 3D islet models. These 3D islet models hold great potential as better experimental platforms to elucidate islet behaviors under both physiological and pathological conditions.

## Microenvironment of native islets

The cell microenvironment plays an important role in maintaining the morphology and the function of islets. Hormones secreted from islet cells balance each other by paracrine, autocrine, or endocrine ways. All these regulation ways depend on the stability of cell microenvironment including the composition of extracellular matrix (ECM) proteins, such as laminin, collagen, and fibronectin. Stable microenvironment is necessary to keep the proliferation, metabolism, and functional activity of islet cells. Abnormal changes of cell microenvironment may influence the gene expression and differentiation of pancreatic cells (Nakamura et al., 2007). In this part, we present the native microenvironment of islets, including cell types, ECM, and their spatial distribution, which are important for engineering functional islet *in vitro*.

### Cell types and their spatial distribution

Normally, the pancreas consists of approximately one million islets in a healthy adult human with each islet almost 200 μm in diameter containing 3000–4000 cells (Ricordi et al., 1988). In the pancreas, islets are composed of at least four types of endocrine cells, including the main composition of β cells distributed in the core of the islets and the other non-β cells (α-, δ-, and pancreatic polypeptide (PP)-cells) distributed at the mantle region. The isolated human islets contain $53.9 \pm 2.5\%$ β cells, $34.4 \pm 2.5\%$ α cells, and $10.4 \pm 0.9\%$ δ cells, whereas the composition of mouse islets has a significant difference where β, α, and δ cells represent $75.4 \pm 1.2\%$, $18.7 \pm 0.9\%$, and $5.9 \pm 0.5\%$ of all the cells, respectively (Brissova et al., 2005). Because β cells are the largest proportion in the islets and its insulin secretion can stand for the islet main endocrine functions, most existing approaches only use β cells to develop simple islet 3D models *in vitro*, such as cell aggregates composed of only

Table 1. Comparison of different methods for fabricating *in vitro* 3D islet models.

| | Cell self-assembly | Hanging drop | Microcontact printing | Microwell | Cell encapsulation | Dielectrophoresis |
|---|---|---|---|---|---|---|
| Islet size (μm) | 100–200 | 100–1200 | 40–120 | 25–210 | 400–1000 | 100–200 |
| Islet uniformity | No | Yes | Yes | Yes | Yes | Yes |
| Co-culture capability | No | Yes | No | Yes | Yes | No |
| Throughput capability | No | Yes | Yes | Yes | No | No |
| Cell density | Low | Low | Medium | Medium | High | Medium |
| Operate complexity | Easy | Easy | Need special equipment and technology | Need special preparation of microwells | Need special material | Need special equipment and technology |

β cells which only secrete insulin. As endocrine cells, α cells secrete glucagon which increases glucose in blood, β cells secrete insulin which decreases glucose in blood, δ cells secrete somatostatin which regulates/stops α and β cells, and PP cells secrete pancreatic polypeptide. A new type of islet cells which secretes ghrelin has also been reported (Wierup et al., 2002), which are termed islet ''ε'' cells. There are only a small number of ε-cells in the normal mouse pancreas (Prado et al., 2004). Several studies have demonstrated that ghrelin could inhibit insulin secretion of β cells and stimulate the glucagon secretion of α cells (Colombo et al., 2003; Qader et al., 2005; Reimer et al., 2003). These endocrine cells influence each other through the paracrine and autocrine communication.

For most clinical and fundamental research, rodent islets and pancreas cells are commonly used. However, it has found out that human islets do not show anatomical subdivisions, and contain fewer β cells and more α cells compared with rodent islets (Figure 1). Normally, islet formation follows an iterative or fractal rule which is universal among mammals (Schneider et al., 1996). However, the spatial distribution of islets in intact mouse pancreas follows a power law with a fractal dimension of 2.1, while in human adult pancreas, it follows a universal power law with a fractal dimension of 1.5 (Jo et al., 2013a,b). Besides, spatial distribution of cells in human islets is like a sandwich with β cells covered by the up-down layer of α cells. Then, the sandwich folds into a complex pattern with circulating vessels (Bosco et al., 2010). Compared with mouse islets, human islets are more heterogeneous in terms of cellular compositions and have more prominent intra-islet vascularity with smooth muscle containing blood vessels (Levetan & Pierce, 2013). Different from human islets, the β cells of mouse islets stay in the core of all the islets, whereas δ cells, α cells, and PP cells distribute along the periphery (Brelje et al., 1989). Another significant difference is that β cells in the mouse islets are only in contact with other β cells, whereas most β cells in the human islets are in contact with α cells, δ cells, or both (Bosco et al., 2010; Brissova et al., 2005; Cabrera et al., 2006). These special anatomical arrangements have significant effects on the cell-to-cell communication within the islet and the precise regulation of hormone secretion in the human islets (Caicedo, 2013).

## ECM

ECM is the extracellular part of multicellular structures that typically provide structural and biochemical support to the surrounding cells, such as cell adhesion and cell-to-cell communication (Gumbiner, 1996). ECM proteins such as laminin, collagen, fibronectin, and E-cadherin are essential to maintain the islet morphology and the islet cell-cell communication. In an islet transplantation study, β cells encapsulated in a hydrogel modified with proteins (e.g. collagen IV, fibronectin, and laminin) showed high viability and functionality (Beenken-Rothkopf et al., 2013). Here, we briefly introduce the relationship between ECM components and the functionality of islets.

Laminins are the major proteins in the basement of membranes, which contain three non-identical chains: α, β, and γ. The laminin molecules are named according to their chain composition. For example, laminin-511, also called laminin 10, contains α5, β1, and γ1 chains (Aumailley et al., 2005). It is present in the human endocrine islet basement membrane. The receptor of the laminin α5 chain also has a strong expression on this basement membrane (Otonkoski et al., 2008). Alternatively, human endocrine islet cells can

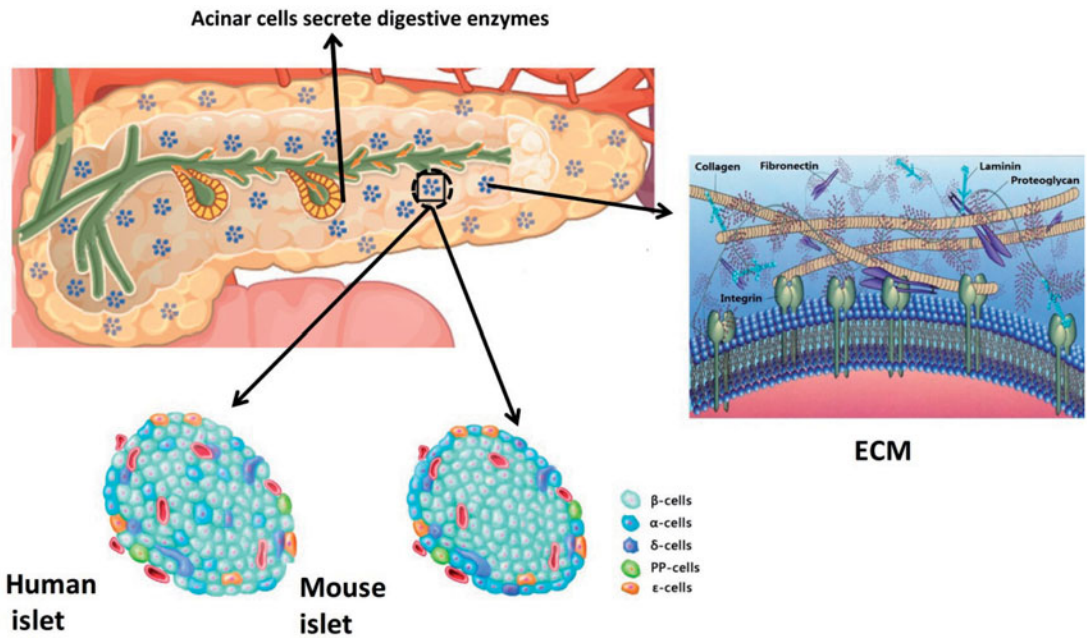


Figure 1. Schematic of islet microenvironment and cells distribution difference between human and rodent islets. The β cells of mouse islets stay in the core of the islets, whereas δ cells, α cells, and PP cells distribute along the periphery. Human islets are more heterogeneous in terms of cellular compositions, with β cells distributing in the periphery of islet like sandwich between two α cell enriched layers. ECM proteins such as laminin, collagen, fibronectin, and E-cadherin are essential to maintain the islet morphology and the regulation of pancreatic cell functionality. Modified from http://greatcourse.cnu.edu.cn/xbfzswx/wlkc/kcxx/kcxx-4.htm.

secrete laminin-332, which is up regulated by stressing conditions (Armanet et al., 2009). Laminin-322 can improve the function of islet cells. At the glucose stimulation, the β cells cultured on the Laminin-332-coated matrix secrete twice more insulin compared with cells cultured on plastic or poly-L-lysine surfaces (Bosco et al., 2000). In addition, laminin promotes the differentiation of pancreatic β cells, as reflected by the expression of laminin in the epithelial basement membrane of the fetal pancreas during β cell differentiation (Jiang et al., 1999). Therefore, the different laminins expressed in the islet cells are all vital for the formation, function, and differentiation of islets.

Collagen, which makes up 25–35% of the total body proteins (Di Lullo et al., 2002), is the most abundant protein in mammals (Burgeson & Nimni, 1992). As the main component of connective tissues, the function of collagen is to support the organ structure and make the tissue flexible. However, many studies have demonstrated that the collagen matrix has the profound function such as promoting the monolayers pancreatic endocrine cell into islet-like organoids (Kadler et al., 2008; Montesano et al., 1983). In a normal human pancreas, collagen deposition is mostly around the large ducts and blood vessels, which is the same for a mouse pancreas. Collagens I, IV, and V are all distributed in the peri-insular region of the human pancreas. Compared with collagen VI, collagen III is stained weakly in human pancreatic tissue (Van Deijnen et al., 1992, 1994). Collagen VI has the double content than collagen I or IV in an adult pancreas, which is a major component of the adult pancreas at the islet–exocrine interface (Hughes et al., 2006). Collagen VI content tends to be age-related increased in total collagen content in human pancreas (Bedossa et al., 1989). Collagen receptors α1β1 and collagens-IV are now known to be important for islet morphogenesis which contributes to the β cell function and glucose homeostasis (Kaido et al., 2004). The cultured β cells adhered to and migrated onto the collagen IV-coated surface, and these responses were mediated almost exclusively by the collagen receptor α1β1. The interaction between α1β1 and collagen IV also resulted in significant insulin secretion. In the presence of collagen IV and laminin, the insulin secretion of β cells was greater than laminin or collagen IV. They work together to promote the survival and functions of isolated β cells (Kaido et al., 2004).

Fibronectin is another glycoprotein component of ECM that binds to membrane-spanning receptors called integrins (Pankov & Yamada, 2002). Similar to integrins, fibronectin binds ECM components such as collagen, fibrin, and heparan sulfate proteoglycans. In the pancreas, fibronectin expresses in the cell clusters budding from the ductal epithelium and the basal membrane of the pancreatic ducts (Cirulli et al., 2000). However, fibronectin plays a negative role in pancreatic cancer, where it protects pancreatic cancer cells from death by inhibiting cancer cells necrosis. The mechanism is through decreased apoptotic DNA fragmentation of cancer cells, which leads to mitochondrial dysfunction and caspase activity (Vaquero et al., 2003). Because the plasma levels of fibronectin correlates to the injury of blood vessels or the loss of endothelial cell polarization, the elevating level of fibronectin has been utilized to describe the clinical symptom of vascular damage. In diabetes patients, the circulating cellular fibronectin significantly increases compared with patients with ischemic stroke or renovascular hypertension. Fibronectin levels in blood may be a predictive marker for endothelial cell activation, especially in diabetes (Kanters et al., 2001). Therefore, fibronectin plays a major role in various cell events, and altered fibronectin expression can induce tumor invasion and vascular lesions of diabetes.

E-cadherin is a key cell-to-cell adhesion molecule, a membrane glycoprotein located at cell-adherent junctions. As a cell surface adhesion protein, E-cadherin establishes and maintains the cell-cell connection such as in the islets of Langerhans. When fabricating β cell aggregates (pseudoislets) *in vitro*, the expression level of E-cadherin can be utilized to indicate the cell junction extent (Bernard et al., 2012). Compared with monolayer culture of β cells, the expression of E-cadherin was greatly enhanced in pseudoislets (islet-like clusters) (Guo-Parke et al., 2012). In human β- and α-cells, E-cadherins express at a similar level and are involved in the maintenance of β cell viability because they protect human β cell from apoptosis (Parnaud et al., 2011). During islet formation, increasing levels of E-cadherin can lower the β cell proliferation by contributing to a decrease of nuclear β-catenin and D-cyclins. Besides, these islets were significantly larger than the control group (Wakae-Takada et al., 2013). The E-cadherin expression levels have impacts on the cell secretory functions. E-cadherin expressed in β cells and δ cells promotes the cell function by sustained or improved insulin or somatostatin secreting level when configured as pseudoislets, whereas α cells lacking E-cadherin expression contain less glucagon (Kelly et al., 2010). Thus, E-cadherin plays a prominent role in the maintenance of islet cell viability as well as it helps to release hormone from the pancreatic islets through cell–cell interactions.

From the above, it can be seen that cell–cell and cell–matrix interactions are critical to islet morphogenesis and hormone homeostasis of islet cells. The ECM protein regulates islet cellular interactions that provide structural information for pseudoislets organization. In the presence of matrix protein, β cells show less apoptosis and higher viability with improved cell functions such as hormone secretion. In a word, ECM is important for the delivery of internal and external information to islet cells, and for the regulation of pancreatic cell growth.

## Microengineering methods for fabricating microscale 3D islet models *in vitro*

Pancreatic islets of Langerhans are the vital organ for the human body to regulate blood glucose. As highly metabolically active mini-organelles, islets are the main focus area for diabetes research. However, experimentally isolated islets cannot really reflect the cell biological character because of rapidly losing cell viability and mass functions (Korbutt et al., 2004). The 3D cell culture gives us an open eye on observing islet cell activity. Different from the cell culture on traditional flat, rigid plastic substrates, 3D cell cultures mimic the native tissue architecture with microtissue geometry, gel-like stiffness, and the complex organization of ECM. Also, it provides the cell–cell and cell–ECM interactions that can better study the biochemistry and mechanics of the islet cells during diabetes research. Numerous studies have demonstrated that 3D islet culture models have higher cell viability, insulin

secretion, and cell–cell connections (Cavallari et al., 2007; Hauge-Evans et al., 1999; Pethig et al., 2008). Here we summarize the microengineering methods that have been developed to fabricate 3D pancreatic islet models *in vitro* and further compare these methods in terms of the parameters of fabricated islets such as islet size, size uniformity, and islet viability.

## Conventional self-assembly method

The most common method for the fabrication of 3D islet models is self-assembly of β cells in either culture on an orbital shaker or static suspension in non-adhesive tissue culture plates (O'sullivan et al., 2010; Weber et al., 2006). Because of its convenience and easiness to operate, this method has been widely adopted to fabricate 3D islet models (Luther et al., 2005; Jia et al., 2007; Persaud et al., 2010).

In this method, the 3D islet models usually attain the size of primary mouse islets (~100–200 μm in diameter) after approximately 3–7 d of culture, containing ~6000 β cells (Luther et al., 2005; Guo-Parke et al., 2012). To achieve better functions of islet cell assembly, one study has demonstrated that rat serum can increase the number of rat cell aggregates and islet diameters, and the insulin gene expression shows a 10-fold increase in comparison with those cultured with fetal bovine serum (Lione et al., 2000). Besides, the 3D islet models fabricated using the self-assembly method exhibited enhanced insulin secretion by 1.7- to 12.5-fold in response to acute stimulation (e.g. glucose, amino acids, incretin hormones, or drugs) compared with equivalent cell monolayers (Guo-Parke et al., 2012). Besides, the glucose-stimulated insulin secretion (GSIS) showed a 6-fold increase in islet 3D models while only 3-fold in monolayer cultures when the glucose concentration was increased from 2 to 20 mmol/L (Chowdhury et al., 2013). Although this method is simple and easy to operate, the aggregation is random, with limited control over the size and the homogeneity of the 3D islet models developed, where large, irregular cell agglomerates often form resulting in decreased cell survivability and functionality due to the limitation of oxygen and nutrition transport (Jo et al., 2013b). Also it is challenging to form aggregates with different types of cells (Figure 2).

## Hanging-drop method

The hanging-drop method is another simple way to construct 3D islet models. The traditional hanging-drop procedure employs a hanging drop of culture media descend due to the gravity and assemble to form a cell spheroid as a microtissue, completely free of contact with any artificial support matrices or surfaces (Figure 2). It has been shown that the volume of reaggregated pseudoislets strongly depends on the cell numbers. A 250-cell aggregate from the average diameter was 95 ± 8 μm (mean ± SD), while for the freshly isolated islets, the average diameter was 122 ± 46 μm. The islet cell loss can be reduced by increasing the glucose concentration or Exendin-4, which is a peptide agonist of the glucagon-like peptide (GLP) receptor that promotes insulin secretion (Cavallari et al., 2007). Generally, islet cells can be re-aggregated into relatively uniform spherical clusters of

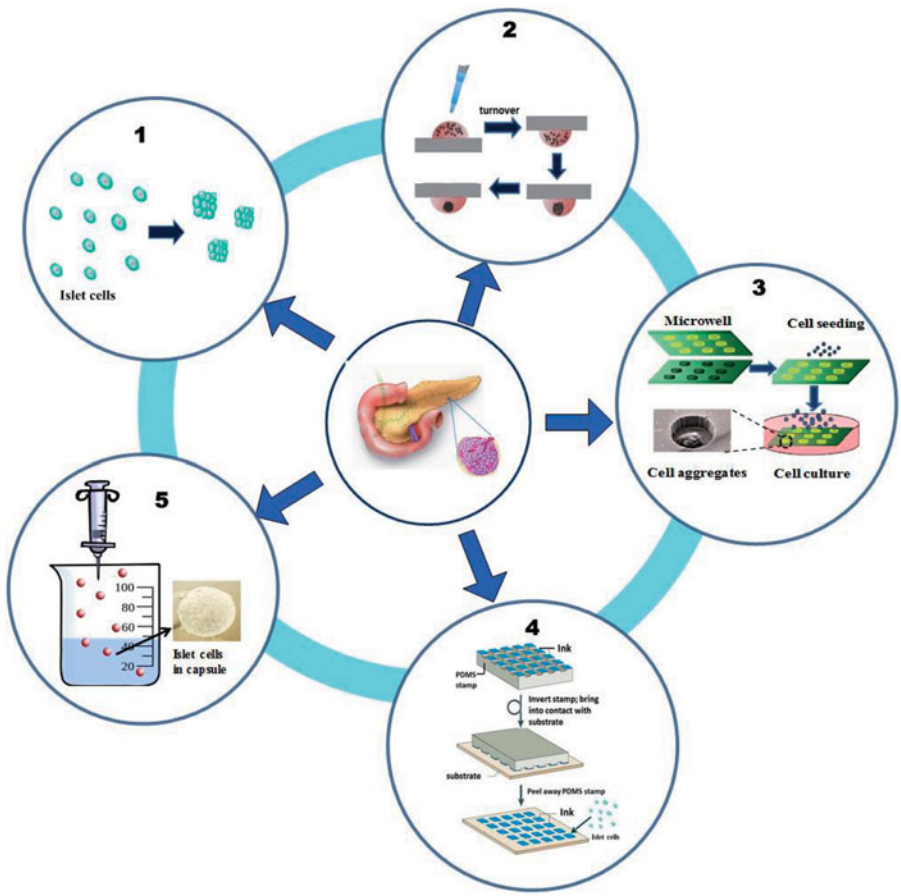


Figure 2. Microengineering methods for fabricating microscale 3D islet models *in vitro*. (1) Conventional self-assembly method; the β cells self-assemble into spheroid in non-adhesive tissue culture plates. (2) Hanging-drop method; a hanging drop of culture media descend due to the gravity and assemble to form a cell spheroid as a microtissue. (3) Microwell-based method; cells drop into the microwells which fabricated by different methods, and then by gravity cells form homogenous spheroids. (4) Microcontact printing; immobilization of cell-adhesive proteins fix to the defined areas and it enhances the aggregation of β cells. (5) Islet cells encapsulation method. The β cells or islet cells encapsulate by a protective membrane like a capsule.

~100 μm in diameter in 30 μl drops (Lione et al., 2000). Cellular composition and structure of the re-aggregated islets were similar to the native islets (Lione et al., 2000).

This method has also been used to fabricate islet models composed of RIN-5F and RIN-m cells (which secrete insulin, somatostatin, or glucagon), where the hybrid islets exhibited an oval shape with sizes ranging from 590 to 1200 μm in 50 μl drops (Jo et al., 2013a,b). Moreover, by using the hanging-drop method, β cell functions such as insulin secretion were almost equal to that of intact islets (Pethig et al., 2008). All these results suggest that the hanging-drop technique has better control of the size and morphology of islet models which are comparable with those of the native islets (Jo et al., 2013a,b; Yip & Cho, 2013). Since the technique does not need expensive equipment and is easy to operate, it has become a favorable choice for the researchers to construct 3D islet models. It provides an easy way to co-culture different types of cells for high-throughput drug screening (Tung et al., 2011). However, the hanging drop method is difficult to control the spatial distribution of different cells in hybrid islet models. Additionally, changing the composition of the culture medium or adding more cells to the aggregates are not feasible with this method. Moreover, this method has a narrow range of islet cell aggregate size and has not shown the flexibility to create different sizes (Banerjee & Bhonde, 2006; Cavallari et al., 2007; Liu et al., 2013; Pethig et al., 2008). These challenges could be addressed by combining the hanging-drop approach with the emerging cell printing technique. For example, uniform-sized embryonic bodies have been formed from stem cells in a controllable manner when integrating these two approaches (Xu et al., 2011), presenting a new option to construct islet models by a simple, robust, and rapid way.

### Microcontact printing

Microcontact printing has been used for the creation of β cell aggregates. Through the microcontact printing process, immobilization of cell-adhesive proteins to the defined areas could enhance the aggregation of cells, which was originally developed by the Whitesides group in 1994 (Figure 2) (Wilbur et al., 1994). In one study, different-sized spots of the cell-adhesive protein–laminin were printed on aldehyde-terminated glass coverslips to induce the formation of a β cell aggregate of different sizes (40–120 μm) (Mendelsohn et al., 2010). The same technique was also used to seed stem cells in circular patches of 120 μm in diameter by using the covalent microcontact-printing of laminin adhere onto glass cover slips. The stem cells then aggregated into clusters and further differentiated into pancreatic endocrine precursors. All these demonstrate that the microcontact printing method can well control the cells aggregate size. It is an efficient way to produce the uniformly clustered functional β cells for diabetes research (Van Hoof et al., 2011).

The microcontact-printing technique allows for the manipulation of aggregate sizes by changing the sizes of the printed area. This technique offers exceptional control over the shapes and sizes of cell clusters and the high-speed printing makes the production of β cell aggregates in a high-throughput manner. Compared with other methods, the size of islets obtained is smaller, but with better uniformity. However, the cell clusters generated using this method could not be removed from the substrates, thus they cannot be used for further medical studies such as some biological tests or implantation. It is also challenging to form co-culture aggregates. In addition, the cell clusters are only two to three cell layers and are not perfect spheroids, thus may not be ideally used as pancreatic islet models.

### Microwell-based method

The microwell method has emerged as a promising method for large-scale cell culture applications, such as for fabricating miniaturized cell-culture wells and forming 3D cell aggregation. A microwell typically has well depths and diameters ranging from tens to hundreds of microns, which can usually hold pico- or nanoliters of liquid. Cells drop into the microwells by gravity and then form homogenous spheroids (Figure 2) (Karp et al., 2007). Compared with other methods, microwell arrays which are quickly fabricated by photolithography technology can be used as miniaturized cell-culture wells and form 3D cell aggregates with well-controlled sizes. The latest study showed that β cell spheroids, prepared using the microwell technique, have significantly enhanced respiratory activity and show a stronger response to glycolysis than those generated using the hanging-drop method. The reason may be the cell density or a change in the metabolic pathway. The genes involved in cellular senescence and glucose metabolism indicated significantly higher values for spheroids obtained using the microwell method (Zhou et al., 2013).

Several studies have indicated the capability of the microwell-based method to control cell aggregation size and uniformity for a variety of cells, including pancreatic islets (from 25 μm to 210 μm in diameter). Compared with suspended β cells, β cell aggregates fabricated using microwells exhibit significantly enhanced insulin secretion (more than four-fold higher) in response to a glucose stimulation (Bernard et al., 2012). The microwell method has been used to form β cell clusters with defined sizes, which was used to measure the dynamics of free-calcium activity ($[Ca^{(2+)}]_i$) and insulin secretion (Hraha et al., 2014). To enhance functionality of 3D islet models, polydimethylsiloxane (PDMS) honeycomb microwell arrays with improved oxygen permeability (80 times as much oxygen as conventional polystyrene culture dishes) have been developed to produce β cell aggregates (Shinohara et al., 2014). Alternatively, co-culture of hybrid cells in the microwells can further improve the β cell function, e.g. 3D spheroids formed by co-culture of hepatocyte and pancreatic islet cells appeared to significantly improve each other's functions especially for insulin secretion (Jun et al., 2013).

With the advances in micro- and nano-technology, various methods have been developed for fabricating microwell arrays with a tailored structure for specific applications. However, there still exist several limitations with this method, such as a narrow range of cell aggregate sizes, special procedures required for the preparation of microwells, and low controllability of cell distribution. Besides, the effect of the physical boundary on cell behavior is still not clear.

### 3D cell encapsulation method

Encapsulating β cells into capsules as an islet model is the most promising way for islet transplantation. The encapsulation method is like a capsule composed of β cells or islet cells, surrounded by a protective membrane (Figure 2). This membrane is semipermeable, only allowing small nutrient molecules and cell-secreted molecules to freely pass through. This can limit the large proteins or cells that may cause the immune response to gain access into the capsules. In these studies, researchers usually obtain islets from the pig pancreas and then encapsulate them for later transplantation. The obvious superiority of this method is that it overcomes challenges of insufficiency in transplantable pancreases donors and avoids the possibility of immune rejection upon transplantation. To fabricate suitable cellular capsules, the key parameters including the capsule permeability, mechanical properties, immune protection, and biocompatibility need to be well controlled. So far, a variety of biomaterials have been explored to improve the biological properties of the capsules, such as Alg-PFC beads to enhance β cell proliferation (Gattás-Asfura et al., 2012), the GLP-1-mimetic peptide amphiphile to stimulate insulin secretion and proliferation of β cells (Khan et al., 2012), gels containing collagen type IV and laminin to enhance the β cells secretion (Weber et al., 2008). Alginate macrocapsules treated by growth hormone-releasing hormone promote the β cells function, such as faster reversal of hyperglycemia and more consistent normal glucose level (Ludwig et al., 2010). All these studies indicate that the encapsulated β cells exhibit excellent viability, reduced apoptosis, and increased insulin secretion.

Generally, each capsule contained approximately 50 000 β cells (Weber et al., 2007), which could remain viable throughout the 3 weeks of culture *in vitro* (Weber et al., 2006). During islet transplantation, a large number of islets (∼15 000–50 000 encapsulated islet equivalents/kg body weight) are needed (Fan et al., 1990; Lum et al., 1991; Sun et al., 1996). This is currently the most promising and well-developed technique for islet transplantation in clinics. Although high cell density can be achieved using this method, which is beneficial for clinical applications such as islet transplantation, it has limited high-throughput capability. Consequently, most of the studies have been focused on the improvement of capsule properties, seldom were applied to medical research due of the complex procedures of β cell encapsulation.

### Other methods

Dielectrophoresis (DEP) is mainly used for cell characterization by measuring the changes in electrical properties. This technique is often utilized for the cell separation and capturation, and more recently, for cell aggregate formation (Flores-Rodriguez & Markx, 2006; Sebastian et al., 2006). For instance, DEP has been used to concentrate insulinoma cells to fabricate 3D cellular constructs (Pethig et al., 2008). Normally, electrofusion-derived human β cells can form static size of 100–200 μm in diameter, corresponding to 6000 β cells. Pseudoislets formation enhanced insulin secretion by 1.7- to 12.5-fold in response to acute stimulation with glucose, amino acids, incretin hormones, or drugs compared with equivalent cell monolayers (Guo-Parke et al., 2012). With the dielectrophoretic-force maintained, these engineered β cell spheroids were able to withstand mechanical shock and fluid flow (Pethig et al., 2008). Furthermore, because no physical contact is required for this method, it has been used to isolate pancreatic islets. With proper voltage and frequency, the islet was separated from the exocrine tissue at the center of the active area of the DEP device. The process was operated on miniaturized lab-on-chip devices (Burgarella et al., 2013). Although the DEP method provides precise control over the cell aggregate sizes, it has limited control over the aggregate size. Besides, specific equipment and experienced professionals are required for DEP, which limits its accessibility.

Alternatively, scaffolds have also been used to fabricate 3D islet models, which can provide signaling interaction between the islets cells and the surrounding ECM. The morphology of scaffolds has great effects on β cell viability and functionality. For instance, small-diameter, densely packed nanofibrous scaffolds can better promote the formation of large, round cell clusters and enhanced insulin production, as compared with large-diameter fibers (Blackstone et al., 2014). In another example, adipose stem cells seeded in a 3D biodegradable scaffold composed of natural polymers dextran and gelatin can effectively differentiate to islet-like clusters expressing islet-specific hormones (e.g. insulin, glucagon, and somatostatin) (Aloysious & Nair, 2013). With the scaffold method, the 3D organization of β cells can be controlled and their functions can be improved through cell–cell interaction by tuning the scaffold architecture. However, the scaffold materials must meet some requirements (e.g. biocompatibility) in order to induce the adhesion, proliferation, and activation of β cells, which is the biggest challenge for the most existing scaffolds. However, unquestionably, compared with other methods, the scaffold method has a more accurate islet structure and is more promising to apply in islet tissue engineering.

Recently, a new method, based on geometric effects of the hydrogel grid structure, presents a different view on the formation of β cell spheroids (Bae et al., 2014). In this method, β cells were encapsulated in hydrogels, which are crosslinked to a hexagonal grid structure. After 13 d' culture, β cells migrated into the empty area of the structure and formed clusters (with a diameter of $83.6 \pm 14.2$ μm) with good morphology and function. The reason for this was that β cell aggregates were strongly promoted by geometric controls, occurring in a combination of biochemical and physiological environments. Another method used a 3D clinostat to simulate microgravity to enhance the aggregation of β cells. By this method, approximately 250 μm size and 100 spheroids per ml of culture media can be obtained. This provides a new method for the reconstitution of a large number of functional β-cell spheroids for diabetes treatment (Tanaka et al., 2013).

## Applications of *in vitro* 3D islet models

Since *in vitro* 3D islet models closely mimic the 3D geometry of native islets, gel-like stiffness, and complex organization of ECM, they bridge the gap between the conventional 2D cell culture system and animal studies. Due to the ethical and cost

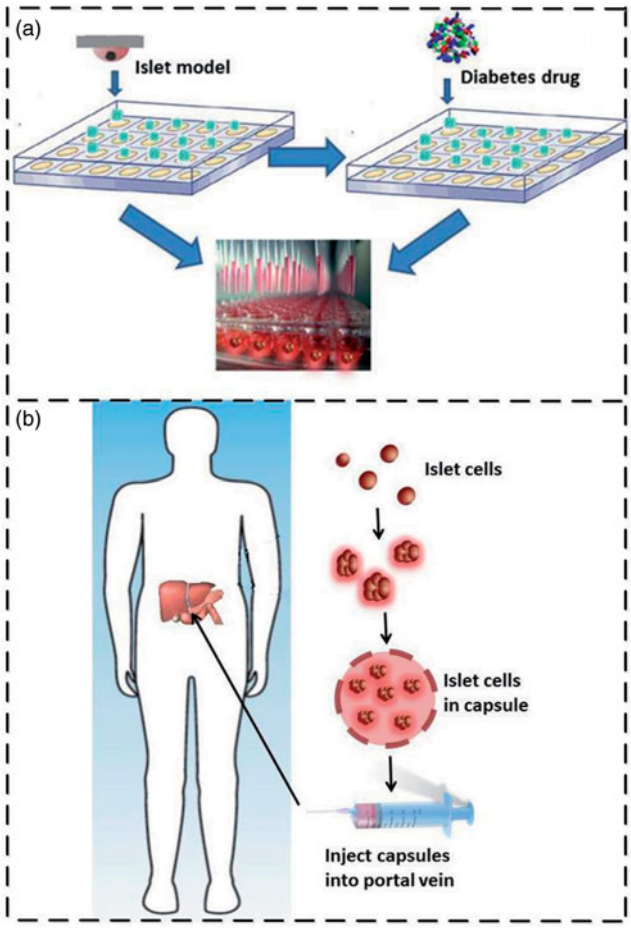


Figure 3. Applications of *in vitro* 3D islet models. (a) Islet model for high-throughput drug screening; by high-throughput fabricated islet models, 3D islet models display a high sensitivity in responding to drugs compared with the conventional 2D models. As a cost-effective approach, it will be used for routine short- and long-term anti-diabetic drug efficacy testing with a high-throughput scale in the future. (b) Islet model for transplantation. After islet cells encapsulated, the cells inject into the portal vein of the body. Encapsulation of pancreatic islets has shown a great potential for clinical treatment by prolonging the survival of cell viability in the absence of immunosuppression *in vivo*. Undoubtedly, it is the most promising way for the treatment of diabetes patients.

advantages over *in vivo* models, the 3D islet models have been used for various biological applications, such as drug screening and transplantation (Figure 3; Kim et al., 2013). Here, we give two examples.

Today, 3D tumor models have been applied into high-throughput drug screening. The obvious reason is that 3D cellular models can better mimic the drug response of native tissues (e.g. toxicity and efficacy) by more closely mimicking the cell physiological microenvironment *in vivo*. For example, *in vitro* 3D TissueFlex® islet model has been reported for diabetic drug efficacy testing (Tung et al., 2011). The authors encapsulated fresh isolated islets in ultrapure alginate and cultured them in TissueFlex®, a multiple, parallel perfused microbioreactor system. The islets cultured in TissueFlex® displayed a high sensitivity in response to drugs and drug dosages. In addition, with an increased number of individuals diagnosed with diabetes, there is a great demand for the development of anti-diabetic drugs which need high effectiveness. Thus, the 3D islet models have become attractive with great potential for high-throughput drug screening. Most importantly, the patterns of insulin release in response to the drug treatment using 3D islet models are in agreement with the data obtained *in vivo* (Li et al., 2013). Besides, numerous tests have proven that the 3D islet models *in vitro* have long-term cell viability (Gazda et al., 2007; Lee et al., 2012), while the isolated islets rapidly lose their mass and viability (Clayton & London, 1996; Olson et al., 2000). The 3D islet models open a new avenue for chronic and accumulative drug testing. As a cost-effective approach, it could be used for routine short- and long-term anti-diabetic drug efficacy testing with a high-throughput scale in the future.

For most islet studies, the ultimate goal is to develop functional islet models for transplantation. With the high prevalence of diabetes, especially for T1DM, islet transplantation is currently the most ideal treatment. Early in the 1986, encapsulation of pancreatic islets has shown great potential for clinical treatment by prolonging the survival of xenografted mice in the absence of immunosuppression (O'Shea & Sun, 1986). The first human clinical trial in encapsulated islet allotransplantation was performed in a 38-year-old T1DM male. The patient was able to discontinue all exogenous insulin treatment at 9 months (Soon-Shiong et al., 1994). In another case, 10 T1DM patients gained stable glycemic control and avoided severe hypoglycemia after injection of islet capsules for 28 months (Ludwig et al., 2013), which is mainly due to the reduced immunological rejection, improved β cells viability, and increased glucose-stimulated insulin release. Insulin and glucagon gene expression were evident in the islets recovered (Sasikala et al., 2013). Alternatively, 3D islet models fabricated *in vitro* have also been tried for transplantation. For instance, pseudoislets formed by reaggregation of the purified endocrine cells from pancreatic islets can delay the process of angiogenesis and revascularization after transplantation (Beger et al., 1998). After the pseudoislets were transplanted into the portal vein of STZ-treated nude mice, the hyperglycemia markedly reduced (Ogata et al., 2004). Undoubtedly, for diabetes patients 3D islet models have demonstrated to be the most promising islet sources for transplantation, although there is still a long way to go.

## Conclusions and future respective

As a relatively independent unit in the human body, the pancreas islet is a mixture of several cell types with a precise spatial distribution and cell proportion. Now, numerous methods have been developed for the construction of islet models *in vitro,* aiming to form β cells aggregates. Many studies have demonstrated that 3D islet models developed *in vitro* exhibit high cell viability and insulin secretion like intact islet as compared with monolayer cell culture, which indicates that these 3D models may bridge the gap between the 2D cell monolayer cultures and animal experiments. It has the great potential to replace monolayer cell culture for diabetes experiment *in vitro*. Furthermore, based on their advantages, they can be used for biofunction assay for diabetes drug screening, pathology model establishing, and islet transplantation for diabetes treatment.

However, the 3D islet models developed with existing methods do not perfectly represent all the structure and

function of native islets. We hope that with the development of bioengineering such as cell printing technology, highly controlled islet micro-tissues with a precise spatial distribution and cell proportion can be realized. Given the great demand for diabetes studies, 3D islet models will eventually become a reliable and an effective platform for diabetes applications. In the future, the improved islet models can be applied in islet cryobiology, high-throughput drug screening, islet transplantation, etc. These all offer the broad prospects for clinical applications.

## Declaration of interest

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