Insulin-secreting β-cell organoids comprise many types of cells, including primary islets, pseudoislets, immortalized cell lines, and stem cell-derived aggregates. Successful maintenance and long-term culture of these β-cell organoids are critical for many translational applications, such as patient-specific disease modeling, drug screening, understanding β-cell physiology, and cell-based therapies to treat diabetes. Due to the mechanical and chemical insult to islets during isolation, islet viability and function are decreased. Partial restoration of the islet microenvironment through engineered biomaterials can improve islet survival and function in vitro culture and in vivo transplantation. Natural and synthetic biomaterials can be engineered to improve the function of β-cell organoids and promote maturation of stem cell-derived β-cell organoids. Improved function and maturation of β-cell organoids will likely lead to improved transplant outcomes, but will also enable better models for physiology, disease modeling, and toxicology studies for type 1 and type 2 diabetes mellitus. The goal of this review is to highlight the diverse array of biomaterials used to enhance the in vitro and in vivo function and maturation of β-cell organoids.

1. Introduction

Diabetes mellitus (DM) is a chronic disease characterized by dysregulation of blood glucose homeostasis due to dysfunction or destruction of the insulin-producing β-cells in the islets of Langerhans. In type 1 diabetes mellitus (T1DM), the autoimmune destruction of the β-cells causes insulin deficiency and hyperglycemia, and patients require lifelong exogenous insulin for management. Type 2 diabetes mellitus (T2DM), insulin resistance in muscle and the liver and impaired insulin secretion from β-cells cause high risk of microvascular and macrovascular complications. Although related, T1DM and T2DM are symptomatically and etiologically distinct. While T1DM is typically associated with severe β-cell deficit, T2DM also has significant loss of β-cell mass approaching up to 40–60% in established T2DM. Combined, T1DM and T2DM accounted for a global estimated cost of US$1.3 trillion in 2015, and prevalence for both is expected to continue to rise over the next decades.

Whereas closed-loop exogenous insulin delivery systems have made remarkable progress in the past decade for treatment of T1DM and T2DM, replacement of the insulin-producing cells through transplantation is regarded as a functional cure. Indeed, clinical pancreatic islet transplantation has evolved over the past three decades to become a realistic treatment option for selected patients with unstable T1DM. However, with only ~1500 patients receiving islet transplants since 2000 with varying levels of success, there is an urgent need to improve both the islet function and supply.

During islet transplantation, the islets are chemically and mechanically liberated from the surrounding extracellular matrix (ECM) and subjected to extended in vitro culture before subsequent transplantation into the hepatic portal vein. This extensive procedure is highly stressful for the islets, which are then subjected to further insult at the transplant site, such as instant blood-mediated inflammatory reaction (IBMIR), low oxygen tension in extrahepatic sites, and host innate and adaptive immune responses. This disruption of cell–cell and cell–ECM junctions during isolation limits islet viability and function. Restoration of these interactions can improve islet survival and function in vitro culture and extensive efforts have been made to re-establish this microenvironment to maintain the function of islets following isolation.

A crucially limiting factor in the widespread implementation of islet transplantation is donor supply. Since the identification of human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) the past two decades have been marked with significant advancements in the generation of a sustainable source of stem cell-derived insulin-producing β-like cells. These human pluripotent stem cells (hPSCs) are capable of generating tissues from all three developmental germ layers, and further differentiating into insulin-producing monohormonal cells that phenotypically and functionally resemble immature β-cells. While very promising, fully functional in vitro differentiation of these hPSCs into mature β-cells remains elusive. 3D aggregation of differentiating stem cells and prolonged engraftment in the in vivo environment have been identified as key promoters of β-cell maturation. This evidence, combined with recent results that engineered biomaterials can promote maturation of other
endoderm organoids, supports the critical role of the islet microenvironment in the directed maturation of β-cells. Recent studies suggest that recapitulation of this microenvironment may play an important role for in vitro β-cell maturation.

Improving the function of isolated islets and directing the maturation of stem cell-derived β-cells necessitate engineered biomaterials that can support these functions. Natural and synthetic biomaterials can be engineered to improve the function and maturation of insulin-producing β-cell organoids, which comprise immortalized cell lines, primary islets, pseudoislets, and stem cell-derived islets (Figure 1). Improved function and maturation of β-cell organoids will likely lead to improved transplant outcomes, but also enable better models for physiology, disease modeling, and toxicology studies for T1DM and T2DM. The goal of this review is to highlight the diverse array of biomaterials used to enhance the in vitro and in vivo function of these β-cell organoids. We further provide perspective of the emerging role of biomaterials to direct β-cell organoid development and maturation.

2. β-Cell Organoids

2.1. Islets of Langerhans

Islets of Langerhans are micro-organs embedded throughout the pancreas that regulate nutrient metabolism by synthesizing and secreting hormones in a highly synchronized and controlled manner. Approximately 1–2 million islets spanning a range of 40–400 μm in diameter require ≈10–20% of the blood flow throughout the pancreas despite accounting for only 1–2% of the pancreatic volume. The human endocrine islet comprises ≈50–75% insulin-producing β-cells, 20–35% glucagon-producing α-cells, 5–12% somatostatin-producing δ-cells, and the remaining endocrine cells comprise ghrelin-producing cells and pancreatic polypeptide-producing cells. In addition to these endocrine cells, these highly sophisticated structures also have a network of accessory cells, including endothelial, neural, and mesenchymal cells that coordinate function and support the high physiologic demand. The organizational architecture and composition of islets are quite heterogeneous and depend on age and islet size, among many other variables that are thoroughly explored in recent studies. Furthermore, rodent and human islets—the two most commonly studied species—exhibit differences in development, composition, and function. However, recent studies have questioned the interspecies variability in islet architecture, and attributed the observed differences to intrapancratic variability among samples. It is important to be aware of this islet heterogeneity when assessing β-cell organoid function and maturation, which are summarized in Table 1.

2.2. Types of β-Cell Organoids

There are multiple sources of cells for β-cell organoids as illustrated in Figure 1. Immortalized β-cell lines derived from mouse (e.g., MIN-6), rat (e.g., INS-1), or human cells (e.g., EndoC-βH1) have been instrumental in understanding β-cell physiology and pathology in reproducible and controlled manners. However, most β-cell lines are problematic, displaying dysfunction in glucose-stimulated insulin secretion (GSIS) and total insulin content, and these cell lines cannot be used clinically. As described earlier, primary islets of Langerhans isolated from the pancreas are the gold standard in studying β-cell physiology. However, the disruption of cell–cell and cell–ECM interactions during the isolation process and broad heterogeneity of islet size have been linked with diminished integrin expression, islet core hypoxia leading to necrosis, apoptosis, and loss of mature β-cell phenotype following isolation. These issues have encouraged researchers to pursue dissociation and controlled reassembly/reaggregation of primary endocrine cells, termed pseudoislets. The size and composition of pseudoislets can be precisely controlled, and numerous studies have demonstrated remarkable improvement in β-cell function in these pseudoislets. Many methods have been developed to maintain small aggregate diameters (<150 μm) and incorporate other cell types, such as endothelial cells, to improve function. Pluripotent and multipotent stem cells differentiated into β-cells in vitro are a sustainable and scalable source of insulin-producing cells for transplantation, but also for the study of β-cell developmental biology, physiology, pathology, and toxicology. Many protocols generate nearly mature β-cells.
from hPSCs using exquisitely tuned multistep combinations of growth factors and small molecules in 2D monolayers or 3D organoids. Some of these well-developed protocols require many weeks (some up to 8 weeks) of in vitro culture to achieve β-cell organoids displaying most mature β-cell specific markers and function.\(^{67–69}\) In addition to hPSCs, other sources of cells derived from tissues, such as fetal pancreatic progenitor cells,\(^{70}\) mesenchymal stromal cells,\(^{71}\) or other progenitor or terminally differentiated cells (e.g., pancreatic ductal cells or hepatocytes)\(^{72,73}\) are differentiated or transdifferentiated in vitro to yield insulin-producing cells.

### 2.3. β-Cell Microenvironment

The islet niche is a highly complex microenvironment of primarily endocrine cells, but also other accessory cells, such as endothelial, neuronal, mesenchymal, and blood cells.\(^{67}\) The islet has two distinct basement membranes (BM), the peri-islet BM and the vascular BM, which are composed of ECM molecules, mainly collagen, laminin, fibronectin, and heparan sulfate.\(^{74}\) Although there are some interspecies structural differences in the BM, the general structure remains the same.\(^{74}\) The peri-islet BM is located at the periphery of the islet and separates the endocrine islet from the surrounding exocrine pancreas and acts as a barrier against infiltrating leukocytes in T1DM.\(^{75}\) The vascular BM surrounds the interpenetrating capillary endothelial cells, and the β-cells are in contact with this vascular BM and are not directly contacting the endothelial cells.\(^{76,77}\) β-cells do not secrete their own BM, and thus rely on secretion of vascular endothelial growth factor (VEGF) to attract endothelial cells that are responsible for depositing the components of the vascular BM.\(^{78–80}\) Because there is relatively little ECM between the β-cells, the β-cells are polarized with integrin activation exclusively at the vascular BM which drives focal adhesions and fusion of insulin granules to this region, with eventual release of insulin into capillaries.\(^{81–83}\) This vascular BM also serves as a reservoir for growth factors that maintain β-cell specific phenotype.\(^{84}\) The homotypic and heterotypic cell–cell contacts that predominate the rest of the islet interior are critical to synchronized insulin secretion.\(^{85–88}\) Such cell–cell contacts include neural cell adhesion molecules (NCAMs),\(^{89}\) E-cadherin,\(^{90}\) connexin 36 gap junctions,\(^{91}\) and EphA-ephrinA signaling.\(^{92}\) The compact nature of islets and the tight coordination between the endocrine cells indicate the importance of three-dimensionality for β-cell function.\(^{48,93}\) Taken together,
cell–cell and cell–ECM interactions are critical to the in vitro and in vivo function of β-cell organoids and should be instrumental factors when engineering advanced biomaterials to support function and promote maturation. For further reading, the role of the islet niche on β-cell function is explored in a few excellent recent reviews.\[80,84,94\]

### 2.4. In Vitro and In Vivo Metrics of Functionality

Although there is significant functional variability between types of β-cell organoids and between species, common assessments of functionality exist in the in vitro and in vivo environments. These assessments and the corresponding techniques are summarized in Table 1. In vitro assessments of function and maturation include analysis of insulin secretion, metabolic activity, viability, markers of β-cell phenotype, and cytokine and hormone secretion. In vivo assessments of function include vascular graftment of the β-cell organoids, systemic insulin secretion, and markers of β-cell phenotype, and cytokine and hormone secretion.

### 2.5. β-Cell Organoid Culture Methods

β-cell organoids are typically maintained in suspension culture that is devoid of most extracellular matrix cues. Static suspension culture entails β-cell organoids on nonadherent surfaces and should be instrumental factors when engineering advanced biomaterials to support function and promote maturation. For further reading, the role of the islet niche on β-cell function is explored in a few excellent recent reviews.\[80,84,94\]

β-cell organoids encapsulated within biomaterials are typically maintained in static culture. However, novel implementation of biomaterials in dynamic suspension or perfused microfluidics may leverage the unique advantages of extracellular matrix cues from biomaterials with the improved mass transport in dynamic cultures.
3. Engineered Biomaterials for β-Cell Organoids

In the present review, the diverse array of natural and synthetic biomaterials is explored in the in vitro and in vivo context, and important design considerations are presented in Figure 2 and Table 2. Specifically, we discuss engineered biomaterials for enhanced β-cell organoid function and maturation of stem cell-derived β-cell organoids. In the in vivo environment, transplanted β-cells are subjected to innate and adaptive immune attacks and must be protected from immune destruction. Decades of research in biomaterials for cell encapsulation or immunomodulation of the transplant site have yielded thousands of papers on this topic, and we direct the readers to recent excellent reviews on islet encapsulation[101–103] and biomaterials for immunomodulation of the islet transplant site.[104,105] While some encapsulation papers will be referenced, the scope of this review will not be focused on immunoprotection.

Furthermore, biomaterials that improve the local transplant site to improve engraftment and vascularization may be referenced; however, we direct the readers to a recent excellent review on engineering the vasculature for β-cell organoid transplantation.[106]

Although pancreatic islets natively exist in a 3D structure, substantial biological insights can be revealed from monolayer culture of β-cells on planar supports[37,107,108] or microcarriers,[109] and single cell encapsulation.[110] Some techniques, such as super-resolution microscopy, which require β-cells to be grown on glass coverslips, enable detailed observation of subcellular processes within the β-cells.[107] Many other researchers utilize 2D ECM substrates for monolayer culture of β-cells to investigate many biological functions, including insulin granule fusion at the basal membrane due to β-cell polarity[82] or endocrine cell fate specification due to cell confinement and integrin activation.[107] This review will not focus on studies involving monolayer culture or single cell encapsulation. However, many of the insights and ECM proteins identified in these types of studies are widely applicable to β-cell organoid culture and frequently translated into biomaterials for 3D β-cell organoid culture, as described below.

3.1. Natural Biomaterials

Naturally derived biomaterials are highly diverse, versatile, and widely used in organoid research.[111] Natural biomaterials, such as protein-based (e.g., collagen[112]) and polysaccharide-based (e.g., alginate[113]) biomaterials, can be chemically or physically modified to provide some control over material properties, such as stiffness, crosslinking density or kinetics, or degradation rates. However, these natural biomaterials are limited by structural and compositional variability, potential immunological

![Figure 2. Summary of tunable parameters for engineering biomaterials for β-cell organoids.](image-url)
responses in vivo, manufacturability, and inability to decouple ligand density from mechanical properties, thus reducing the customization and tunability for a finely controlled biomaterial system. Nonetheless, the biological similarity and multifunctional complexity of natural biomaterials provide for a permissive and biologically relevant environment to explore β-cell organoid development and function.

### 3.1.1. Engelbreth–Holm–Swarm Matrix

Extracts of Engelbreth–Holm–Swarm (EHS) matrix, variants of which are commercially available as Matrigel, Geltrex, or Cultrex BME, are a mixture of basement membrane proteins and growth factors purified from the EHS tumor. When incubated at 37 °C, EHS matrix extracts polymerize to create a 3D scaffolding gel for the culture of many cell types. Composed primarily of laminin, collagen IV, nidogen, and perlecan, EHS matrix also contains nearly 2000 other unique proteins in various concentrations depending on the lot. Although EHS matrices have been extensively used in many applications, including metastasis and cancer cell growth, morphogenesis, and organoid generation, this matrix suffers from lack of clinical translatability due to its tumor-derived nature, batch-to-batch variability, compositional complexity, and inability to easily tailor the biophysical or biochemical properties.

Notwithstanding, EHS matrix has been instrumental in understanding islet and β-cell organoid function. 3D EHS matrices have been used for long-term culture and maturation of many primary progenitor or fetal cell sources, such as fetal porcine islets, fetal murine pancreatic progenitors, and fetal human pancreatic tissue. Combined with forced expression of transcription factors and finely tuned timelines of differentiation factors in culture media, EHS matrices have also facilitated the 3D culture and conversion of pancreatic ductal cells into insulin-secreting β-cell organoids and organoids of other pancreatic lineages. Matrigel-coated tissue culture plastic (TCP) and flat Matrigel slabs have also enabled the generation of hPSC-derived β-cell organoids. In both instances, hPSC-derived β-cells were cocultured with endothelial cells to facilitate the important interactions that exist between β-cells and endothelial cells. EHS matrices also improve β-cell organoid function. Murine islets exhibit improved static GSIS and viability when embedded in Matrigel in vitro, and Matrigel improved intramuscular engraftment of murine islets indicated by quicker return to euglycemia and improved IPGTT results. Murine islets in Cultrex BME demonstrated upregulated integrin α3 expression, upregulated phospho-FAK (focal adhesion kinase) expression, and protection from anoikis.

### 3.1.2. Decellularized Biomaterials

Decellularization of tissues and organs is a process in which cells are lysed and rinsed from the surrounding extracellular matrix, leaving behind a complex biological scaffold for use in tissue engineering applications. The complex 3D ultrastructure of the ECM and tethered or sequestered growth factors are preserved during this process, leaving behind an intact bioactive scaffold with most biophysical and biochemical cues still present. Most cells and organoids are reperfused or seeded onto these scaffolds, and the cells migrate into the scaffold. Many decellularized tissues have been used as scaffolding for β-cell organoids to improve in vitro insulin secretion, increase metabolic activity, and facilitate long-term culture, while other scaffolds are used to restore normoglycemia in extrahepatic in vivo environments. These tissue sources are derived from human, murine, and porcine sources, and include pancreas and pericardium, and liver. Decellularized tissues have also demonstrated positive enhancement of maturation for differentiating stem cells into β-cell organoids. Scaffolds derived from liver pancreas and spleen have all increased the insulin (or c-peptide) expression of stem cell-derived β-cell organoids compared to 2D culture platforms on TCP.

Decellularized tissues can also be formed into engineered hydrogels by further solubilizing the scaffold into its protein
monomeric components. These components can then be manipulated to form hydrogels with various crosslinking times and stiffness by controlling the salt concentration, pH, and temperature. Further control of the crosslinking kinetics can be modulated by incorporating glycosaminoglycans (GAGs) or proteoglycans (PGs). While the 3D architecture of the native tissue is not preserved in these ECM hydrogels, they offer enhanced tunability of many parameters, such as better control of component concentration and gelation kinetics. Pancreatic tissue, lung, and bladder have been used as ECM-based hydrogels for long-term culture and maintenance of insulin secretion from islets. However, some of these decellularized tissues led to atypical islet inflammatory activation.

### 3.1.3. ECM-Derived Biomaterials

Further control of the ECM composition and structure is achieved using matrices comprising purified ECM proteins or protein fragments, such as collagen, laminin, fibronectin, etc. Collagen I and IV are the most abundant collagen molecules within the endocrine pancreas and support β-cell structure and function. Many collagen-based biomaterials have been used to improve in vitro islet function and in vivo engraftment, and have been nicely reviewed. Collagen I, III, and IV scaffolds have demonstrated improved islet and β-cell organoid viability in long-term culture and increased β-cell gene expression compared to suspension culture. Furthermore, type I collagen scaffolds with 5% chitosan and laminin yielded improved islet viability and increased GSIS. Collagen IV incorporated into microporous poly(lactide-co-glycolide) (PLG) scaffolds significantly improved islet metabolic activity, stimulation index, and viability, and decreased apoptosis. These islet-loaded collagen/PLG scaffolds were then transplanted into the epididymal fat pad of mice, and the collagen IV promoted significantly earlier restoration of normoglycemia. Collagen scaffolds are also populated with other cell types in addition to islets, such as endothelial cells or fibroblasts, to improve in vitro islet viability and insulin secretion and promote faster in vivo engraftment leading to reduced critical islet mass required for normoglycemia restoration. For differentiation of stem cell-derived β-cell organoids, 3D scaffolds of collagen I blended with Matrigel markedly improved insulin secretion. Collagen V/Matrigel substrates also demonstrated significant improvements in iPSC differentiation into β-cell organoids, yielding upregulation in key pancreatic transcription factors and hormone expression, and enhanced GSIS.

Gelatin, a biopolymer derived from the partial hydrolysis of collagen, is also used extensively for organoid encapsulation and many other tissue engineering applications. In immortalized cell line culture, fine control of gelatin fiber diameter and spacing can control 3D aggregation and insulin production from the β-cell organoids. In a unique layer-by-layer coating technique with alternating coatings of gelatin and fibronectin, mouse β-cells formed tightly formed spheroids with increased GSIS, upregulated glucose transporter genes, and upregulated expression of connexin 36 gap junctions for improved synchronization of insulin secretion. Gelatin has also been grafted with proteins necessary for differentiation (i.e., Activin A), which allows for continued presentation of the protein during hPSC differentiation and improved differentiation efficiency to the pancreatic progenitor stage. Gelatin can also be blended with other biopolymers, such as dextran, to provide a 3D scaffold environment for the differentiation of adipose-derived stem cells into glucose-responsive insulin-secreting β-cell organoids.

### 3.1.4. Alginate

Alginate is a natural anionic polymer refined from seaweed, and it is the most commonly utilized polymer for islet encapsulation research. Alginate is a block copolymer of β-1,4-mannuronic acid (M) and α-1,4-guluronic acid (G) residue blocks in different sequences, and the ratio of M and G blocks determines the crosslinking kinetics, degree of crosslinking, and swelling of the crosslinked hydrogel. Divalent cations, such as Ca2+, Sr2+, and Ba2+, preferentially bind the G blocks, and therefore, the rigidity and stability can be increased by increasing the ratio of G blocks or increasing the cation concentration. Because alginate lacks bioactive ligands that are necessary for cell anchoring, many groups have tethered bioadhesive ligands into the alginate or blended the alginate with ECM molecules to improve cellular viability and function. Alginate IV and VI blended into alginate that is tethered with laminin-derived adhesive peptides has significantly improved metabolic activity of encapsulated islets, improved viability, enhanced GSIS, and reduced cytokine-mediated cytotoxicity. Alginate hydrogels can also be fabricated into many structures, such as threads, microcapsules, or sheets, to support islet viability and function in vivo for many months.

Alginate has also been used to influence the in vitro differentiation and in vivo function of hPSC-derived β-cell organoids. Chemically modified alginate, such as triazole-thiomorpholine dioxide (TMTD) alginate, mitigates the fibrotic response when implanted into the intraperitoneal space, and can support the long term function of hESC-derived β-cell organoids, indicated by normoglycemic glucose levels and successful IVGTT. Undifferentiated hESCs encapsulated in alginate and then undergoing differentiation in the capsules demonstrated significantly improved c-peptide protein synthesis (20-fold) compared to 2D culture. In a similar study, this same group demonstrated that increased capsule stiffness increases differentiation efficiency to the definitive endoderm stage (DE) but significantly suppresses efficiency of pancreatic progenitor (PP) production. Alginate encapsulation during the stages following pancreatic progenitor phenotype suggests that cell confinement can promote differentiation toward an islet-like profile. These three studies indicate that 3D scaffolds are important for β-cell organoid generation and that the stiffness should be finely tuned to achieve the desired efficiency of various stages in β-cell differentiation.

### 3.1.5. Fibrin-Based Matrix Biomaterials

Fibrin is a fibrous matrix protein derived from the proteolytic cleavage of fibrinogen by thrombin and subsequent
polymerization that occurs during blood clotting. This biodegradable protein’s primary function is clot formation for wound healing, but its crosslinking abilities have been leveraged for islet encapsulation and islet transplantation, which are summarized in a recent review. Fibrin has protective effects against H2O2, which is a macrophage-secreted factor during the in vivo inflammatory response. Islets encapsulated in fibrin were protected from H2O2-mediated damage, and the fibrin preserved GSIS and islet morphological integrity. The fibrin scaffold can also be supplemented with hypoxia-mitigating factors, such as emulsified perfluorodecane (PFD), that supplement the matrix with oxygen and support increased islet GSIS and viability. β-cell organoids derived from human umbilical cord stem cells, bone marrow-derived mesenchymal stem cells (MSCs), and hESCs have all been cultured in fibrin matrices to aid in differentiation and maturation. These studies have relied on the 3D support provided by fibrin to promote improved differentiation (increased transcription factor expression and insulin expression) compared to unencapsulated or 2D culture. β-cell organoids derived from rat insulinoma lines (INS-1) also exhibited increased GSIS, upregulation of integrin avβ3 and phospho-FAK, and decreased apoptosis, supporting the observation of the improved benefits of 3D culture of β-cell organoids.

Fibrin scaffolds can also be fabricated from plasma separated from whole blood. Some groups use plasma instead of purified fibrinogen, and subsequently polymerize the scaffold by introducing thrombin to cleave the fibrinogen within the plasma. Plasma is rich in growth factors and enzymes and lacks standardization in preparation and application, which can introduce variability between batches. However, plasma is frequently used autologously in islet transplantations in rodents, non-human primates (NHPs), and humans, thus lessening the immunological response to the polymerized fibrin graft.

3.1.6. Silk Biomaterials

Silk is a highly versatile biopolymer that can be processed into many formats, including fibers, films, scaffolds, hydrogels, and microparticles, for cell encapsulation. Further tunability of the biopolymer chains can be engineered, such as altered surface properties through chemical modification of the amino acid side chains, incorporation of cell recognition sequences through genetic engineering, tunable stiffness through fiber concentration, or various degradability rates based on silk fiber processing techniques. 3D silk scaffolds support long-term viability, metabolic activity, and function (improved GSIS) of primary islets and insulinoma β-cell organoids compared to unencapsulated controls. For enhanced control of adhesive ligand presentation, ECM-derived bioadhesive motifs can be inserted at the N-terminus of silk proteins to enhance pseudo-islet aggregation of dissociated primary islet cells or insulinoma cells. Incorporation of other cell types and proteins into the silk scaffold may further improve β-cell organoid function. For example, MSCs and ECM proteins (collagen IV and laminin) encapsulated with primary islets into the silk hydrogels significantly improved in vitro islet gene expression, GSIS, and lowered expression of islet dedifferentiation genes.

3.2. Synthetic Biomaterials

Compared to naturally derived biomaterials, synthetic biomaterials offer enhanced reproducibility, scalability, greater control over composition, and tunable and independent control over biodhesive ligand presentation, mechanical properties, and degradation. The chemically defined microenvironment provided by synthetic biomaterials allows for precise control over many matrix properties for organoid culture, as illustrated in Figure 2. Many synthetic biomaterials are functionalized with ECM-derived cell adhesive peptides that are chemically defined, have diverse functions, and can be conjugated to the biomaterials with great tunability and precision. However, these synthetic mimics often fall short of their native ECM counterparts and fail to achieve the diverse functionality of the whole protein, such as high affinity receptor binding and exposure of cryptic binding sites upon mechanical activation or proteolytic degradation. Nonetheless, these tunable synthetic biomaterials can be engineered with versatile modularity to target specific cell–cell and cell–ECM interactions and eliciting signal transduction cascades to direct morphogenesis, differentiation, and function of cells. A wide range of synthetic biomaterials have been used to mimic the complex microenvironment of β-cell organoids for enhanced in vitro culture and in vivo engraftment.

3.2.1. Poly(ethylene glycol) (PEG) Biomaterials

PEG hydrogels comprise one of the most used synthetic polymers in tissue engineering and regenerative medicine applications. PEG hydrogels are highly versatile owing to the wide range of polymer architectures (linear, multiarm star) and molecular weights, ease of derivatization, resistance to biofouling and nonspecific protein adsorption, flexibility in utilizing various crosslinking platforms, and ability to functionalyze with many biological ligands. Many PEG hydrogels have also been engineered with dynamic control of biochemical and biophysical properties to engineer a dynamic microenvironment for organoid culture and development. PEG chains are typically terminated with various functional groups, such as maleimide, norbornene, or acrylate that enable control over crosslinking chemistries, crosslinking efficiencies, and reaction kinetics.

PEG hydrogels have played a critical role in identifying key adhesive ligands necessary for β-cell adhesion that promote survival, function, and differentiation. Because PEG lacks cell adhesive binding sites, short peptide sequences from ECM proteins, identified primarily via competitive adhesion assays and mutagenesis experiments, are frequently tethered to the PEG chains to provide a bioactive and cell instructive microenvironment. For example, laminin-derived and collagen-derived adhesive peptide sequences were covalently tethered into a 3D PEG hydrogel to improve β-cell survival, decrease apoptosis, and increase GSIS. Full ECM proteins entrapped with the PEG matrix also support significantly increased insulin secretion, decreased apoptosis, and increased survival of both islets and immortalized β-cell organoids. However, the 3D environment of PEG without any adhesive ligands
or proteins can also encourage cell–cell contacts within β-cell organoids and improve viability and long-term survival.[217,218] Other peptides/proteins have been tethered into PEG hydrogels to improve in vitro insulin secretion, such as glucagon-like peptide-1 (GLP-1), which is a potent insulinotropic and antiapoptotic hormone,[219,220] and cell–cell signaling fusion protein, EphA/EphrinA.[221,222]

For differentiation experiments, pancreatic progenitor cells encapsulated in unmodified PEG survived and differentiated into immature β-cell phenotype characterized by nonglucose responsive insulin secretion.[223] However, when collagen type I is entrapped within these 3D PEG scaffolds, the pancreatic progenitors differentiate into more mature, glucose-responsive β-cell organoids, thus demonstrating the importance of cell–matrix interactions in β-cell maturation.[224] In addition to encapsulation, PEG-based hydrogels (termed Amikagels due to polymerization of amikacin, an aminoglycoside antibiotic, with PEG-diglycidylether) facilitated spontaneous spheroid formation of hESC pancreatic progenitors by promoting spontaneous aggregation.[225] The pancreatic progenitors and endothelial cells were seeded on top of the Amikagel, and the subsequent spheroids demonstrated enhanced differentiation efficiency (improved GSIS and c-peptide expression) toward a mature β-cell phenotype.[225]

The protein and peptide tethering capabilities of PEG have also enabled its versatile in vivo use to improve the β-cell organoid engraftment. VEGF tethered to proteolytically degradable PEG hydrogels[226,227] has induced robust angiogenic and vascular responses that enhance in vivo islet graft function and reduce marginal islet mass required for normoglycemia.[228–230] PEG has also been blended with Matrigel in islet encapsulation to improve the physiological function and survival in highly vascularized transplant sites.[231]

3.2.2. Polyester Biomaterials

Polymers containing ester groups in the backbone are generally used for their biodegradation and ability to incorporate other growth factors and ECM proteins.[201] Commonly used polyesters for cell encapsulation are derivatives of poly(lactic acid) (PLA, PDLA, PLLA, PDLLA), poly(glycolic acid) (PGA), poly(lactic acid-glycolic acid) (PLGA), and poly(caprolactone) (PCL). Microporous PLG scaffolds are often created using a NaCl particulate which is leached out to allow for infiltration of islets or β-cell organoids.[232] These scaffolds are also loaded with various proteins or peptides that are released over many days to weeks to aid in engraftment or β-cell organoid function. Examples of such proteins or peptides include transforming growth factor-β1 (TGF-β1) used to modulate the local in vivo immune environment,[233] exendin-4 used to prolong islet engraftment in the epididymal fat pad,[234] or exendin-4 used promote in vivo maturation of hESC pancreatic progenitors indicated by increased c-peptide expression.[235] These islet-loaded PLG scaffolds have also been adsorbed with collagen IV to promote rapid restoration of normoglycemia in diabetic mice and prolonged function.[236,237]

3D printing of PLA scaffolds provides the mechanical support and desired macroporous architecture that is necessary for efficient oxygen diffusion and eventual graft retrievability.[217] PLA scaffolds were 3D-printed and filled with a fibrin scaffold containing stem cell-derived β-cell organoids. These scaffolds supported efficient delivery of oxygen to the β-cell organoids, provided a retrievable scaffold from the subcutaneous space after 12 weeks, and supported long-term function of β-cell organoids.[217] PCL scaffolds have also been successfully used for long-term delivery systems for islets into extrahepatic sites, such as the epididymal fat pad.[238]

3.2.3. Polyvinyl Biomaterials

Vinyl-based polymers are an attractive choice for many β-cell organoid transplantation applications due to the low immunogenicity, cytocompatibility, and nondegradable nature.[201] There are a number of vinyl polymers used in tissue engineering applications, such as poly(2-hydroxyethyl methacrylate) (PHHEMA), poly(vinyl alcohol) (PVA), poly(N-isopropylacrylamide) (PNIPAM), poly(vinylpyrrolidone) (PVP), and poly(acrylamide). Mechanical stiffness can be tuned in poly(acrylamide) hydrogels, and this was leveraged to demonstrate that compliant, nonfunctionalized poly(acrylamide) wells promote increased GSIS from β-cell organoids compared to stiff microwells.[239] This stiffness-dependent insulin secretion was subsequently shown to be regulated though myosin light chain kinase (MLCK) and Rho-associated protein kinase (ROCK) mechanosensing pathways.[239]

PVA can be fabricated in numerous ways for tissue engineering applications, such as homogenous hydrogels or electrospun fibers, and is also frequently combined with many natural or synthetic biomaterials.[240] Electrospun, nanofibrous PVA scaffolds have been used to provide a 3D scaffold environment for differentiating iPSCs into β-cell organoids. These fibrous PVA scaffolds have contained PLLA[241] or platelet-rich plasma and human adipose-derived MSCs.[242] The in vitro cultured β-cell organoids express key β-cell transcription factors and increased c-peptide expression compared to 2D culture.[241,242] PVA hydrogels have also shown to be useful in long-term cryopreservation and successful thawing and recovery of macroencapsulated islets.[243]

3.2.4. Other Synthetic Biomaterials

Polydimethylsiloxane, also known as PDMS, is a silicone-based polymer most commonly used in microfluidic applications, but increasingly being used in cell scaffolding and drug delivery applications.[244] Macroporous PDMS scaffolds, fabricated with NaCl particulate leaching to achieve pore sizes between 200 and 450 μm, have been utilized to provide a mechanically stable and retrievable islet scaffolding that allows for enhanced host vascularization at the transplant site.[245] PDMS scaffolding can also be supplemented with dexamethasone, an anti-inflammatory glucocorticoid, to mitigate local inflammation at the islet graft site and improve early engraftment of the islets.[246,247] β-cell organoids are highly metabolically active in vitro and in vivo, and require supplementation of oxygen within large macroencapsulation devices. Engineered oxygen-generating
bimaterials have been developed to address this issue,[248,249] and PDMS has shown to be an excellent biomaterial for slow release of oxygen for many weeks. Solid calcium peroxide encapsulated within PDMS discs provides controllable generation of oxygen, and these discs (termed OxySite,[255]) have been used for long-term, stable generation of oxygen for islets in vitro culture and in vivo macroencapsulation.[250–252]

Manipulating the nanotopographical environment can regulate cellular functions and direct stem cell fate.[253,254] Gradient nanopatterned chips composed of polystyrene recently enabled the identification of a pore nanotopography (200–300 nm diameter) that promoted the aggregation and efficient differentiation of β-cell organoids from hPSCs.[255] These stem cell-derived aggregates were small (≈50 μm diameter) and expressed some markers of immature and mature β-cell phenotypes, but glucose responsiveness was not reported.[255] Nanotopography manipulation can also promote long-term function of islets. For example, zirconia substrates with nanoscale roughness promote long-term in vitro culture of human islets and prevent apoptosis and dedifferentiation compared to smooth zirconia substrates.[256]

4. Conclusions and Outlook

Successful culture of insulin-producing β-cell organoids is critical for many translational applications, such as patient-specific disease modeling, drug screening, understanding β-cell physiology, and maintenance and transport for cell-based therapies to treat diabetes.[257,258] Many studies demonstrate that recapitulating the extracellular microenvironment maintains β-cell organoid function, improves viability, and enhances in vivo engraftment. The diverse array of biomaterials presented in this review further support that the cell–cell and cell–ECM cues are necessary for β-cell function. Continued progress in understanding the intricacies of the β-cell microenvironment should be pursued by studying the cell–cell and cell–ECM interactions of the native islet in situ. Furthermore, there is a need for more investigation of the heterogeneity of β-cells within the islet,[259] heterogeneity of intrapancreatic islet composition and architecture,[260] as well as interspecies variability in islet morphology.[261] Exploring these aspects of islet biology will enable researchers to engineer better biomaterials to target particular functions and behaviors of β-cell organoids and further direct maturation of stem cell-derived β-cell organoids.[261]

Current in vitro protocols for stem cell-derived β-cell organoids are making substantial progress toward achieving a mature β-cell phenotype. Many strategies, such as modulating the duration of stages, the concentrations and types of pathway inhibitors and activators, cluster size, or circadian rhythms, have been recently developed to achieve a more mature phenotype in vitro culture.[69,68,69,262] One hurdle in achieving this mature phenotype is the lack of standardized comparative assessment of the mature β-cell phenotype due to varying quality of human islet preparations, which are frequently batches rejected for clinical use.[13] In vivo maturation of β-cell organoids is considered a “black-box” with relatively little insight into the key mechanisms that regulate this maturation.[12,263,264] Indeed, transplanting immature β-cells is clinically fraught. Current in vitro protocols generate many immature proliferating β-cells[265] and undefined non-endocrine cells that have the potential to form teratomas.[266,267] However, strategies are being developed to provide fail-safe approaches to prevent tumorigenesis, such as incorporating suicide gene casettes into the genome.[268]

Based on the evidence presented in this review, the β-cell microenvironment is critical to function and recapitulating the key parameters may be necessary to achieve a fully mature β-cell phenotype prior to transplantation. Integrin-mediated adhesion signaling is critical in islet development,[269,270] and this could provide the basis for engineering biomaterials to direct maturation of β-cell organoids. Furthermore, recent evidence demonstrates that integrin-mediated mechanosignaling can direct fate decisions of pancreatic progenitors in vitro.[37] Integrins can also be used to identify β-cell-committed cells during differentiation. For example, integrin subunit αl (CD49a) is preferentially expressed in immature β-cells, and can be used to purify differentiating endocrine cells to produce highly enriched β-cell organoids.[269]

Many groups have utilized engineered biomaterials to direct the fate and maturation of stem cell-derived organoids.[111,271,272] For example, fully synthetic PEG hydrogels engineered with fibronectin- or laminin-based adhesion moieties can support expansion and differentiation of intestinal stem cell organoids,[134,273] and further promote engraftment and healing of murine colonic mucosal wounds.[148] Biomaterials are also being developed for many other human organoid models, including liver, stomach, intestine, pancreas, lung, and brain.[127,275] Many of these novel organoid culture strategies, combined with the knowledge of biomaterials for islet function, may provide the inspiration needed for development of advanced biomaterials to direct β-cell organoids toward a fully mature phenotype.

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Conflict of Interest

The authors declare no conflict of interest.

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