Encapsulation of Stem Cells in Research and Therapy

Isgard S. Hueck, Jane Frimodig, Pamela Itkin-Ansari and David A. Gough

Abbreviations

ECM	Extra Cellular Matrix
hESC	Human Embryonic Stem Cell
iPSC	Induced Pluripotent Stem Cell
LPC	Liver Progenitor Cell
MSC	Mesenchymal Stem Cell
PPC	Pancreatic Progenitor Cell

1 Introduction

Diseases of the liver and the pancreas are on the rise worldwide (World Health Organization, 2017). The liver and the pancreas are the two organs most responsible for metabolic regulation and, when impaired, have dramatic effects on patients' lifestyles and survival rates [1, 2]. As of 2013, there were more than 30 million Americans [3] and 29 million Europeans suffering from chronic liver diseases [4]. Chronic pancreatitis ranges from 12 (US) up to 120 (Asia) people in each 100,000 [2, 5]. The survival rate of pancreatic cancer is only 8%, which makes it the most lethal cancer worldwide (World Health Organization, 2016). The prevalence of Diabetes cases has risen from 108 million in 1980 to 422 million in 2014 (World Health Organization, 2016). Both, pancreas and liver diseases are increasing to an epidemic level [2, 6], and more than 17,000 people are on the waiting list for liver

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transplants in the US, of which more than 1500 people die every year while waiting for a transplant organ (American Liver Foundation, 2016). Thus, there is a desperate need for alternative therapies to organ transplantation.

The pancreas and liver develop from the same pool of endodermal progenitor cells [7]. However, expandable long-term primary cell cultures for implantation are not available for either hepatocytes from the liver or endocrine cells from the pancreas. Therefore, another cell type has to be used if cell therapy is to be a feasible alternative to organ transplantation: e.g. stem cells. A therapy that would allow implantation of stem cells to help repair or substitute for organ functions could ultimately reverse the dire prognosis for patients with liver or pancreatic disease. Stem cell therapies could be the key for both short-term approaches until a donor liver is available, or for long-term solutions to mimic the endocrine function of the pancreas by providing in vivo production of insulin.

Since stem cells were discovered, their potential to differentiate into replacement cells for regenerative therapies has been discussed as a cure for many diseases. However, the dynamic play between factors that influence stem cell differentiation, and/or the ability to secrete therapeutic substances in vivo, is very complex and not fully understood. Furthermore, pockets where stem cells can survive, differentiate, or secrete proteins need to be protected in a foreign host environment to avoid the need for chronic immunosuppression. An alternative is cellular encapsulation intended to avoid rejection by the immune system and to control growth of cellular implants in a defined space. Encapsulation techniques for cellular implants, ranging from single cells to complex structures of organoid tissues, are engineered as an immunoprotective packaging tool for stem cell survival in vivo. This chapter will summarize and examine the advances of stem cell encapsulation approaches in research and therapy. Selected examples for stem cell encapsulation devices will be discussed with focus on the treatment of chronic and acute liver diseases and Diabetes Mellitus.

2 History of Cell Encapsulation

By the early 1900s, the idea of isolating cells via encapsulation was an appealing scientific method for research. In 1912, Murphy and Rous developed a method of implanting tumor cells in a chicken embryo inside a chicken egg [8]. After carefully patching the egg's shell using paraffin under sterile conditions, the chicken egg resembled a perfect encapsulation device to keep the implanted cells alive. The cellular environment was contained inside the eggshell, yet the implanted cells were protected from mechanical destruction or immune responses. About 20 years later, in 1933, the scientist Vincenzo Bisceglie, at the Royal University of Bari in Italy, followed up on the encapsulation technique using chicken eggs. He discovered that an immune response is lacking in chicken eggs until the 18th day of embryo development [9]. Therefore, his implanted cells, encapsulated in chicken eggs, grew rapidly for about 18 days before an immune response arose. In his experiments,

Dr. Bisceglie used sarcoma and carcinoma cells, and a tumor mass was able to develop for 2½ weeks of protected growth in the egg capsule. In further cell encapsulation approaches, Dr. Bisceglie used a collodion bag to hold cells for implantation in vivo [10]. The collodion bag was prepared of material synthesized of gelatin and nitrocellulose treated carefully in a procedure with alcohol, ether, and hot and cold water. In these studies, Dr. Bisceglie used mouse tumor cells contained in the collodion bag, which functioned as a protective semipermeable membrane, for implantation into the abdominal cavities of guinea pigs. It was reported that the tumor cells survived for 12 days in the guinea pig's abdomen [10]. This was the birth, not only of cellular encapsulation techniques, but also of modern cancer research with tumor studies in foreign hosts. However, the implanted cells were originated from aggressive growing tumors and did not have any regenerative effects on the host. Another 30 years passed before cell encapsulation was considered as a tool for tissue engineering in regenerative therapy.

In 1964, Thomas Chang introduced the first polymer membrane encapsulation of aqueous solutions of proteins. He developed stable capsules up to 100 μm in diameter that were protected from immunoreactions, and were capable of delivering therapeutic enzymes in vivo [11]. However, until a more biocompatible material was developed and other issues were addressed, cell and tissue encapsulation was not ready for clinical applications in humans. The idea of encapsulated cell replacement therapies for humans was driven by Diabetes Mellitus research, which was highly impacted by advances of Dr. Ernst Pfeiffer in the 20th century [12]. In the 1970s and 1980s, several research groups working in the field of diabetes were pioneers in experiments with the transplantation of pancreatic *Islet of Langerhans* cells, the endocrine tissue that releases insulin [13]. Furthermore, Australian Dr. Kevin Lafferty laid the groundwork for encapsulation of cell therapies by exploring immunoreactions to implants [14]. American Dr. William Chick used primary islet cells for encapsulation techniques in pre-clinical studies with small and large animals [15]. Drs. Lim and Sun were the first to do microencapsulation of primary islet cells in diabetic rats [16]. At the same time, Dr. Robert Langer was recognized as the first researcher to integrate biology and material sciences for cellular encapsulation techniques [17, 18]. Not until 1994, however, were encapsulated vital islet cell implants successfully used in humans. A study conducted by Drs. Soon-Shiong and Heinz et al. is recognized as the first clinical trial using alginate encapsulated human islets in one diabetic patient [19]. The diabetic and end-stage kidney diseased patient was reported to be insulin-free for 9 months after cell implantation [20]. Although this was a great first success, later clinical studies using this approach, did not confirm insulin-independence in patients with diabetes [21]. In 1999, a German scientist group, led by Dr. Mathias Löhr in Berlin, used cellulose sulfate polymers in clinical trials as an innovative strategy to embed cells in micro-capsules for treatment of patients with end-stage pancreatic cancer [22–24]. Since then, many variations of embedding materials, either of natural and biocompatible components, synthetized polymers, or a mixture of thereof have been tested to encapsulate cells for regenerative therapies.

In recent decades, the discovery of stem cells for cellular replacement therapies made the encapsulation approach a crucial engineering tool. Stem cell populations can be uniquely sensitive in their need for a specialized environment in order to survive and differentiate to therapeutically useful cells in vivo. Encapsulation materials with specific characteristics for filtering molecules that may pass through the encapsulation layer have been developed in the past 20 years [25–31].

This chapter will review how the combination of two developments has advanced the prospect for cell therapy. Encapsulation materials and designs, used as an engineering tool for device development, and the understanding of stem cell manipulation, may provide a promising approach to the treatment of liver diseases and Diabetes Mellitus.

3 Background

3.1 Stem Cells for Therapeutic Use

Currently, expandable long-term primary cell cultures are not possible for either hepatocytes from the liver or endocrine cells from the pancreas. Therefore, another cell type has to be found if cell therapy for the liver and pancreas is to be scalable and feasible. One possibility is stem cells. There are several different types of stem cells that can be utilized for cellular implantation devices to treat chronic diseases [32]. Depending on the clinical application, stem cells for use in regenerative therapies include *embryonic stem cells (ESC), induced pluripotent stem cells* (iPSC), adult *mesenchymal stem cells* (MSC), and *progenitor cells* (PC). Although stem cells can develop into various functional mature cells, this chapter is focused on examples of liver and pancreas cell development.

Embryonic stem cells (*ESCs*) originate in the embryonic blastocyst, are pluripotent, carrying the potential to differentiate into any cell of the body, and can proliferate in vitro. They are usually cultivated on feeder cell layers or matrigel, so that a sufficient quantity of the proper cell type is produced prior to implantation [33]. However, this process is still limited in scale. There also may be a risk of introducing murine viruses from matrigel [33]. Human embryonic stem cells (hESC) can be manipulated to differentiate into hepatocyte-like cells, as well as beta-cell-like cells that produce insulin in vitro and in vivo. HESCs have been used in cellular implantation devices, as will be described later in this chapter. However, the use of embryonic stem cells has been a subject of ethical debate ever since their discovery. Furthermore, immune responses from the implant host need to be addressed in transplantation approaches that include hESCs.

Another stem cell type used in encapsulation devices is *induced pluripotent stem cells, or iPSCs.* These cells are usually derived from adult terminally differentiated cells that have been genetically altered to introduce pluripotency. The first human iPSCs were originated from adult human fibroblasts genetically reprogrammed with

viral vectors [34]. Since then, other easily accessible cells have been used for induction of pluripotency, such as keratinocytes from a plucked hair, peripheral blood cells, and epithelial cells excreted in urine [35–37]. Although these cells are readily available, the process of reprogramming creates challenges for use in humans. Disadvantages of iPSCs include low efficiency in the rate of successful conversions, risk of inserting genomic mutations into the cells, and high tumorigenecity [38]. However, iPSCs are free from immune and ethical issues that are associated with ESCs. IPSCs have shown promise insofar as they can be differentiated to hepatocytes and hold potential for autologous transplantation in liver disease treatments [39, 40]. Still, use of iPSCs entails worries about the use of viral vectors, changes in cell cycle proteins, and the origination of teratomas. Development of efficient non-viral reprogramming techniques, better biological understanding of reprogramming effects on the epigenetic state, and certified stocks of iPSC clones, will all be critical to clinical use of iPSCs in regenerative therapies [41, 42].

Adult stem cells of mesodermal origin *(MSC)* can be isolated from bone marrow, adipose tissue, and other sources [43]. They remain undifferentiated throughout most of their life, resting in adult tissue until needed. However, in cell culture, they lose their proliferative capacity over time. Although they are *adult* stem cells, MSCs can be stimulated to differentiate into hormone-secreting cells as they are found to express human insulin, glucagon, and somatostatin in immunocytochemistry experiments [44]. MSCs are shown to exhibit insulin-production in vivo in diabetic mice [45] and seem to improve insulin sensitivity in peripheral tissues. MSCs can be genetically modified to express a variety of therapeutic factors; therefore they can serve as a promising platform for cell-based microencapsulation. MSCs are known to be hypoimmunogenic. In vitro and in vivo studies revealed that encapsulated human MSCs showed a significant decrease in cytokine expression, compared to other encapsulated stem cells, because they do not express certain surface receptors involved in immune responses [46]. Encapsulated hMSCs were shown to maintain their mesenchymal surface markers and can differentiate to all typical mesoderm lineages. However, MSCs lack the high proliferative potential that ESC's and iPSCs ensure [47].

Progenitor cells (*PC*) are adult stem cells that are generally able to differentiate, but are limited to the cell types of their resident organ. They are still in the process of being defined for both the liver and the pancreas. Generally, use of ESC or iPSC involves a differentiation step to committed liver progenitor (LPC) or pancreas progenitor cells (PPC) before they can be implanted. Terminal differentiation to the desired mature cell type then takes place in vivo. Figures 1 and 2 show a schematic overview of stem cells used in encapsulation therapies for the liver and pancreas, as well as their differentiation potential.

In summary, disadvantages of iPSCs and ESCs include ethical issues and possible mutations. However, advantages of these stem cell therapies are the strong proliferative potential of iPSCs and ESCs, as well as their capacity to differentiate into any desired cell [47]. On the other hand, MSCs are widely available and have excellent hypoimmunogenicity to keep immune responses low. PCs are specific to

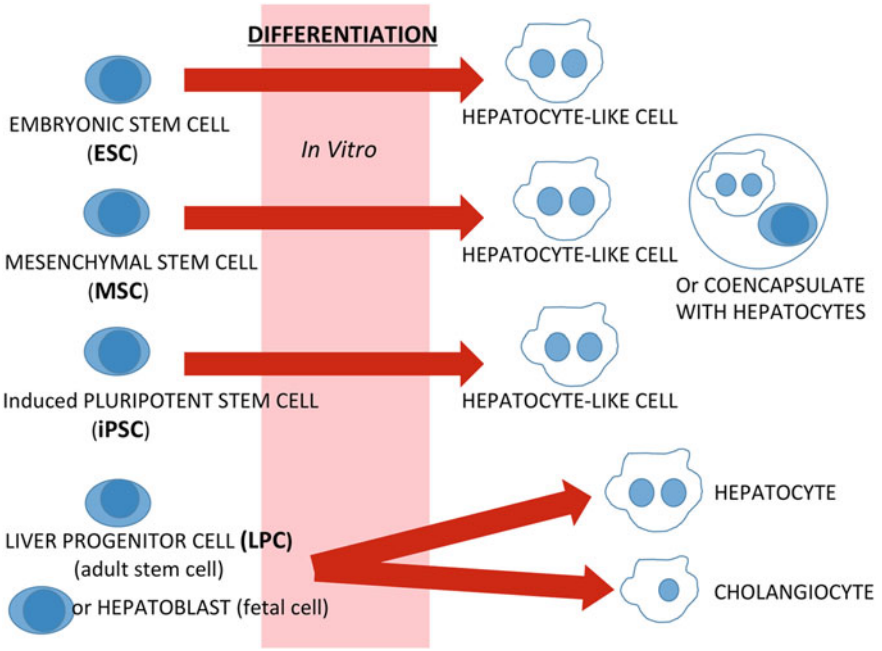

Fig. 1 Stem Cell Differentiation in the Liver. Embryonic Stem Cells (ESCs), Mesenchymal Stem Cells (MSCs), and induced Pluripotent Stem Cells (iPSCs) can differentiate to Hepato-Like Cells in vitro. Co-encapsulation with Hepatocytes enhances the effects of stem cell regenerative therapies. Liver Progenitor Cells (LPCs) and Hepatoblasts are bipotent. LPSs are generally a differentiation step from stem cells to committed organ-specific cells. Hepatoblasts are human fetal cells from early gestation and are extremely limited as a cell source

their organ of origin. The choice of the cell type used in encapsulation devices is affected by the clinical application and the desired therapeutic outcome. The beneficial effects of encapsulated stem cell therapies may not be restricted to cell restoration, but stem cells may also provide therapeutic use due to their delivery of proteins in vivo, as they actively contribute to their environment by secreting cytokines, growth factors and extracellular matrix (ECM) molecules that act either on themselves (autocrine actions), or on neighboring cells (paracrine actions) [48].

3.2 *Types of Cell Encapsulation*

Many approaches have been made to cure disease by implantation of whole donor organs, organ parts, or functional units of organs (organoids), down to single cell implantations. Depending on the desired function of the implant, the implant size, the choice of implanted cells, and the encapsulation material, may vary.

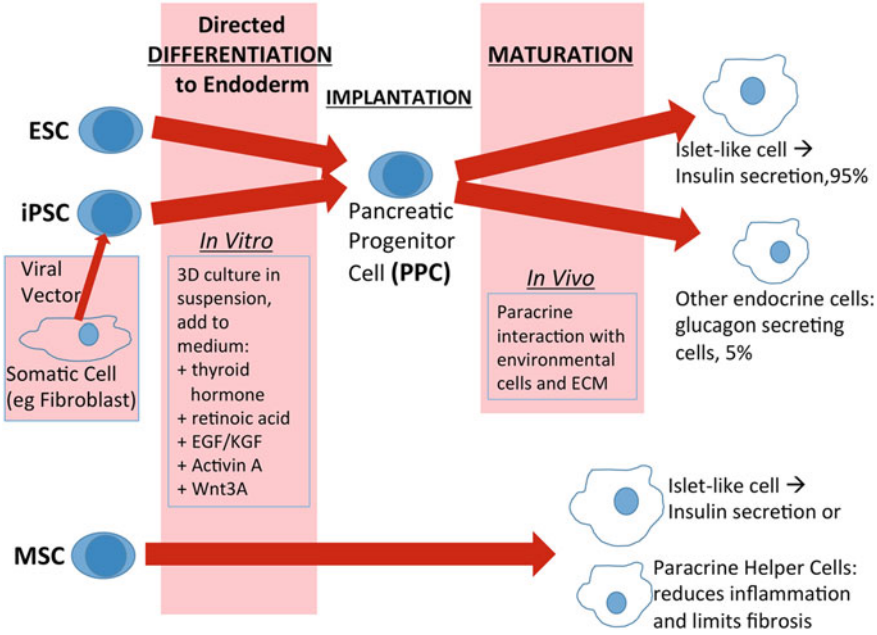

Fig. 2 Stem Cell Differentiation in the Pancreas. Embryonic Stem Cells (ESCs) or induced Pluripotent Stem Cells (iPSCs), after they were transduced with a viral vector, can differentiate to Pancreatic Progenitor Cells (PPCs) in vitro. PPCs then mature into endocrine islet-like cells in vivo under certain circumstances. Mesenchymal Stem Cells (MSCs) can develop into paracrine helper cells if encapsulated

The choice of cell type used in an implantation device depends on many factors, such as the availability of cells or clinical need. Generally, there are 3 types of cells used for cellular therapy: *autologous* cells, *allogeneic* cells, and *xenogeneic* cells [49]. *Autologous* cells are derived from the same patient and eliminate any immune reactions. However, depending on the nature of the specific cells, these may be limited in availability since a lack of healthy cells might cause the disease in the first place. New approaches, such as developing cellular therapies from frozen umbilical stem cells, collected at birth, may fill the gap of healthy autologous cells in the future. *Allogeneic* cells are derived from another donor and are controversial due to the transmission of viral diseases and immunoreaction concerns [50]. *Xenogeneic* cells are derived from another species and are known to trigger strong immunoreactions due to inter-species differences [51]. However, transgenic pig islet cells have been used in experiments to treat Diabetes Mellitus [52–54]. Though xenogeneic cells are easily accessible, the danger of viral disease transmission still remains, as the example of porcine endogenous retrovirus infections has shown [55]. In the age of gene manipulation, genetically engineered cells, either from allogeneic or xenogeneic origin, have become promising encapsulation cells [56].

Encapsulated stem cells can be engineered to differentiate in a desired manner, to secrete a specific substance, or to express fluorophores for visualization in vivo.

Another choice in cellular therapies is the cell encapsulation method. Cell encapsulations can be *permanent* or *degradable*. If permanent, most likely the cell is being implanted for purposes of secretion of some required factor, e.g. insulin. The implanted cells are restricted by its encapsulation so that cell-cell contact cannot be made with host cells. On the other hand, if the encapsulation material is degradable, the implanted cells will eventually be able to differentiate and incorporate with its host tissue, e.g. for tissue repair therapies. However, all implants face challenges of immunoreactions, mechanical damage, containment of cells, control of growth and differentiation, and mismatch of microenvironmental factors. Numerous engineering techniques of the encapsulation method have been tailored to help achieve the desired outcome. These techniques are generally classified as *microencapsulation* and *macroencapsulation* [57].

Microencapsulation usually refers to small spherical capsules or conformally coated vehicles of single cells or small cell clusters. The size of microcapsules can vary from 0.3 to 1.5 mm. The small capsule sizes provide an advantage for mass transport considerations due to the large surface-area-to-volume ratio, and the spherical form is a relative stable shape. Microcapsules are generally highly biocompatible as a consequence of hydrophilic properties of the encapsulating material [57]. It has been shown that smaller capsule sizes seem to trigger less cellular immune response in foreign hosts [58]. Traditional microcapsules contain cells in a hydrogel core surrounded by a membranous structure for stability. Microbeads without a distinct membrane have been successfully used for certain applications. For example, the encapsulation of three-dimensional aggregates of pluripotent stem cells in soft alginate micro-beads (so called embryoid-body cells) have shown promising results in regenerative therapies for liver cell re-growth [59].

Macroencapsulation devices deal with larger living cell populations or part of tissues that are physically isolated from directly interacting with the surrounding host tissue. The implanted cells are typically encapsulated by a 2-dimensional membrane of natural or synthesized material that varies in structural, functional, and mechanical properties [57]. While the microenvironment inside the encapsulated compartment can be varied, these devices still rely on the host's homeostatic mechanisms for control of pH, metabolic waste removal, and nutrients (extravascular supply). Therefore, the association with the host's vasculature plays an important role in the success of implanting macroencapsulation devices. To sustain the viability of the encapsulated cell mass, extravascular devices rely on the formation of new blood vessels (angiogenesis) at the host-device interface following implantation. Angiogenic stimulation in the host, applied in combination with the use of encapsulated stem cell populations, have made it possible to keep functional cell organoids alive for prolonged periods of time following implantation into various body cavities or tissues. While preventing direct contact with host cells, the encapsulation material can also be engineered to minimize adhesion properties

on the implant's surface, or to selectively filter certain sizes of molecules for transport through the encapsulation layer. By engineering specific properties of the encapsulation material (e.g. chemical affinity to certain molecules or pore size), immune responses to the implant can be minimized, while at the same time, angiogenesis or tissue regrowth through paracrine factors can be stimulated around the implant site. An example of a macroencapsulation device using cellular therapy for treating Diabetes Mellitus is discussed in the pancreas section (part 5) of this book chapter.

3.3 Therapeutic Reasons for Encapsulation of Stem Cells

(a) Immunoprotection and Paracrine Effects

The foremost reason for cell encapsulation is immunoprotection, designed to isolate the implanted cells from attacks by host immune cells. The encapsulation layer prevents the direct cell-cell contact of implanted cells with host cells. However, paracrine effects, as a form of neighboring cell-to-cell communication, still need to be transmitted through the encapsulation layer in order to alter the behavior of the cells inside the encapsulation layer, as well as cells of the surrounding host tissue. With careful design, this effect can be therapeutically used to induce changes in cells, either on the encapsulated cells to secrete certain substances, or on host cells surrounding the implant to stimulate re-growth of tissue or new vascularization (angiogenesis). In order to allow transport of oxygen, water, and necessary nutrients to enter, and metabolic waste products to exit the encapsulation chamber, the encapsulation layer must be semipermeable [60]. However, circulating larger host molecules (e.g. certain immunoglobins like IgM) or immune cells can be prevented from contact with the cells inside the implantation chamber [61]. Figure 3 shows a model of necessary barrier properties and molecules traveling in and out of an encapsulated chamber containing implanted cells.

It is essential that the encapsulation layer controls the rate of therapeutic products exiting the encapsulation chamber. It has been shown that different cell types have unique metabolic requirements; therefore the encapsulation layer has to be optimized for each cell type according to pore size or other membrane properties [57].

Furthermore, if a specific secretion product from implanted cells is desired (e.g. insulin), the secreted protein must be able to enter the blood system of the host and be distributed throughout the host's body quickly. Therefore, blood vessel growth towards the implant is essential to provide short diffusion distances necessary for secreted proteins to enter the blood system of the host. Growth of new vascularization surrounding the cellular implant is initiated by paracrine effects of angiogenic growth factors, either produced by the implanted cells, or as a result of

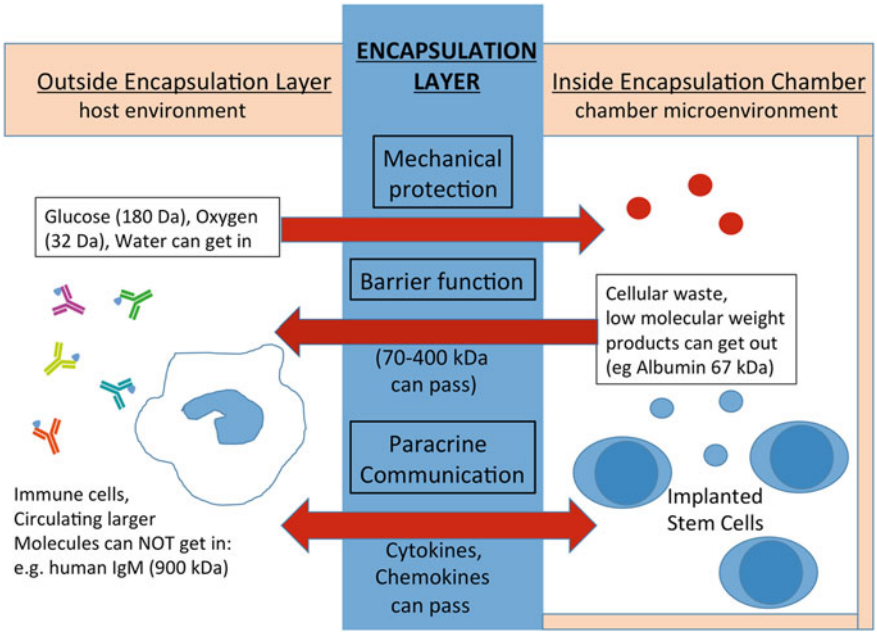

Fig. 3 Barrier Functions of the Encapsulation Layer. The semipermeable membrane of the device encapsulation layer controls the traffic in and out of the encapsulation chamber. Necessary nutrients for cell survival, like glucose, oxygen, and water, as well as cellular waste products are small enough to pass, whereas immune response cells of the host are prevented from immunogenic sampling of the implanted cells due to their larger size. Paracrine communication between the encapsulated cells and the host is still attained through the layer

engineered properties of the encapsulation layer [62]. An example of angiogenic growth around a cellular implantation device post explantation is shown in the pancreas section of this book chapter (part 5).

(b) Microenvironment

As the significance of the microenvironmental role in cellular survival has become more obvious in recent years of research, designing specific characteristics of the environment around stem cells has become an essential tool in the engineering of encapsulation devices. Since the extracellular matrix (ECM) around encapsulated cells in the chamber can be engineered in regard to the nature of the biomimetic scaffold, it's architecture, and the presence of growth factors, as well as recognition motifs, implanted cells can be enabled to exhibit specific functionality in vitro and in vivo [63]. Furthermore, encapsulation material can be manipulated to replicate cell-cell interactions, cell-matrix interactions, and chemical gradients. Approaches to engineering the cellular microenvironment for stem cells have been explored by (1) providing a 3D environment for cell growth, (2) co-culturing of cells (usually with non-parenchymal cells), (3) using cellular genetic modifications, (4) modifying

the ECM in encapsulation chambers, and (5) adding growth factors and other signaling moieties [63–65].

The ECM represents an essential player in the stem cell niche environment, since it directly influences the maintenance, proliferation, self-renewal, and differentiation of stem cells. During human embryonic development, the ECM acts on embryonic stem cells to alter their gene expression and induce their proliferation or differentiation needed for the development of tissue. In adult humans, adult stem cells are maintained in a quiescent state until the surrounding microenvironment actively signals the need for self-renewal or differentiation to form new tissues [66].

The physiochemical composition of the ECM is critical to the regulation of stem cell fate; this includes paracrine factors, cytokines, chemokines, glycoproteins, and oxygen tension. These factors will affect important properties, such as mechanotransduction, the effect of mechanical forces on the cell. Furthermore, the ECM can promote interactions between stem cells, neighboring differentiated cells, and/or adhesion molecules [67]. To mimic certain conditions of the ECM in encapsulation devices, biophysiochemical factors, such as composition, shape, topography, stiffness, and mechanical strength, can be modified to control stem cell behavior. Various studies involving different types of scaffolds that regulate stem cell fate by mimicking and altering ECM properties have been done [68]. The complex interactions between stem cells and their niche creates the dynamic system necessary for repairing tissues, and for the ultimate design of stem-cell therapeutics [69].

The main advantage of implanting encapsulated stem cells is that cues, not only from the direct microenvironment around the stem cells, but also from the host tissue and/or the encapsulation material, can serve to direct the differentiation of the stem cells into precisely the kind of cells desired, in order to optimize therapeutic effect. Cues can be designed to work either to maintain proliferation and stem cell phenotype, or to direct differentiation to mature cells. Cues can be added in vitro, before cells are implanted, incorporated during the encapsulation process, or initiated by the implant microenvironment. For example, ways to control behavior-initiating signals to stem cells are enhanced by 3D cell culturing, cell-cell signaling as in co-cultures, use of stimulants from the ECM, by growth and diffusible factors, and by genetic engineering [63]. Therefore, engineering tools, like cell manipulation and the design of encapsulation material, are critical in determining cell fate of stem cells living in a foreign host.

Another important factor to consider is the geometry of the implant. Many years of in vitro cell culturing, most commonly performed in a 2D environment in flasks or plates, demonstrated that growth and behavior of cells outside their natural 3-dimensional environment is not ideal in 2D layers. The optimal shape for *microencapsulation* implants is a sphere, due to maximal surface area and to complement diffusion limits of oxygen (about 200 μm through tissue). For *macroencapsulation* approaches, the optimal implant device contains a 3D interior growth chamber to provide a microenvironment that is specific to the needs of the implanted cell population. The effects of 3D cultures and co-cultures seem to be particularly important in regenerative therapies for liver diseases, which is further discussed in the liver section (part 4) of this book chapter.

(c) Cell Containment and Control of Growth and Differentiation

Cell encapsulation naturally provides mechanical protection of cell populations during the implantation process, as well as from contractile forces from adjacent tissues during movement of the host's body, when implanted. Cell therapy devices are usually implanted in body cavities or soft tissue areas; however everyday movement can shift the implants around. Furthermore, cells themselves have the tendency to migrate through tissue and may try to escape from the implantation device with amoeboid movement, driven by motogenic signals from cytokines, or cell-cell interactions of their environment [70]. The mechanisms that govern the movement of eukaryotic cells are still not fully understood because of the dynamic nature of their regulation through biochemical and mechanical interaction that control cell motility [71]. Physical properties of the ECM and the encapsulation layer, such as rigidity, porosity, topography and insolubility, are factors that influence various mobility-related cell functions, like cell division, tissue polarity, and cell migration [72]. Therefore, the encapsulation layer of a cell therapy device needs to be designed to prevent cellular protrusion, in order to prohibit cell escape from the chamber and to keep host immune cells from antigenic sampling of the implanted cells. The encapsulation layer, in combination with microenvironmental factors, needs to protect the encapsulation chamber from cellular depletion, while also preventing over-exuberant stem-cell proliferation [72]. A carefully designed microenvironment, as well as the encapsulation layer itself, both play a specific role in the proliferation rate and overall fate of the implanted cells. Encapsulation material can be engineered in such a way as to either help retain stem cells in an undifferentiated pluripotent state, or to direct their differentiation in a desired manner. Modifications of alginate encapsulation materials, using peptides on the surface of the encapsulation wall, were observed to control the proliferation and rate of differentiation of certain encapsulated cells [73]. Several studies demonstrate that surface modifications containing nano-particles in the cell-encapsulation material allow control over growth and cellular differentiation [74, 75]. Cell-to-cell communication, as initiated in co-encapsulation with different companion cells, is also influential in directing the stem cell's differentiation. A specific example, discussing beneficial versus unwanted cell behavior due to co-culturing of liver cells with MSCs, is discussed in the liver section (part 4) of this book chapter.

3.4 Materials for Cell Encapsulation

Encapsulation material can be either a *naturally occurring* substance or a *synthetic* polymer. Both types can be modified further to improve desired properties of the encapsulation layer. Important properties of encapsulation material include biocompatibility, pore size, material density, stiffness, and material degradability [63]. For manufacturability considerations, the cost and reproducibility, as well as the ease of use in clinical settings, are also essential. Engineering techniques in the

design of implantable biomaterials is a large field of research by itself. For the purpose of this chapter, only the most widely used materials for cellular encapsulation therapies will be discussed.

Naturally occurring encapsulation materials include alginate, chitosan, collagen, gelatin, agarose, and combinations of cellulose sulfate. Synthetic materials include PEG (PolyEthyleneGlycol), PLGA (PolyLacticGlycolicAcid), PVA (PolyVinylAlcohol), PU (PolyUrethane), and PTFE (PolyTetraFluoroEthylene); all are polymers with elastic properties and variable biodegradability [76]. For example, PTFE is not biodegradable and, as many synthetic materials, lack recognition motifs in a cell environment, but can be modified by means of chemical engineering and nano-engineering techniques [63, 76]. Most cellular encapsulation approaches use a combination of a natural and a synthesized component to achieve the desired encapsulation properties and cellular responses in vivo. The speed and sterility of the process, and the quality and purity of the encapsulation agents, are critical for success and reproducibility of the product. Use of non-toxic manufacturing procedures contribute further to the viability of the cells, when implanted in humans or animals. Impurities of the encapsulation material may influence cell viability and function, including the rate of production of the therapeutically relevant molecules secreted by the encapsulated cells [77].

Alginate is by far the most common material used for stem cell encapsulation due to its early discovery, excellent biocompatibility, ease of availability, and low cost. Alginate, extracted from seaweed, can either be used by itself, or in combination with a synthetic polymer such as Poly-L-Lysine. Alginate has a high permeability, but is fairly weak in physical strength and stability. Its softness is advantageous for hydrogel-based encapsulation of cells. For some applications, such as the embryoid-body cells, the flexibility of pure alginate is a beneficial characteristic, as small membrane-less microcapsules are filled with 3-dimensional cell clusters [59]. This is known as conformal encapsulation. Figure 4 shows an example of experimental alginate microbeads used to determine optimal cell density for implants. Encapsulated cells are engineered to express fluorescent signals for visualization purposes. The production of uniformly sized microcapsules, and the use of biocompatible cross-linking reagents for alginate capsule stabilization, are critical factors for successful microbead production (see Fig. 4).

Alginate capsules are often coated with Poly-L-Lysine (PLL) in order to improve mechanical strength of the encapsulation. However, PLL lacks biocompatibility [78]. This can be corrected by adding another thin alginate layer on the outside of the PLL-alginate capsule. The triple layer construct is called APA (Alginate-Poly-L-Lysine-Alginate). Unfortunately for use in long-term applications, alginate exhibits issues with low durability over time. The stability of alginate decays relatively fast, which can lead to cracking of the alginate layer and increased porosity of the capsule after a short implantation time. Consequences of a porous alginate layer are the exposure of the encapsulated cells to immune responses of the host, and may result in the failure of a long-term implant [79]. Furthermore, some studies suggest that even ultrapure alginate may contain endotoxins and polyphenols, which could produce an inflammatory response in vivo [80, 81].

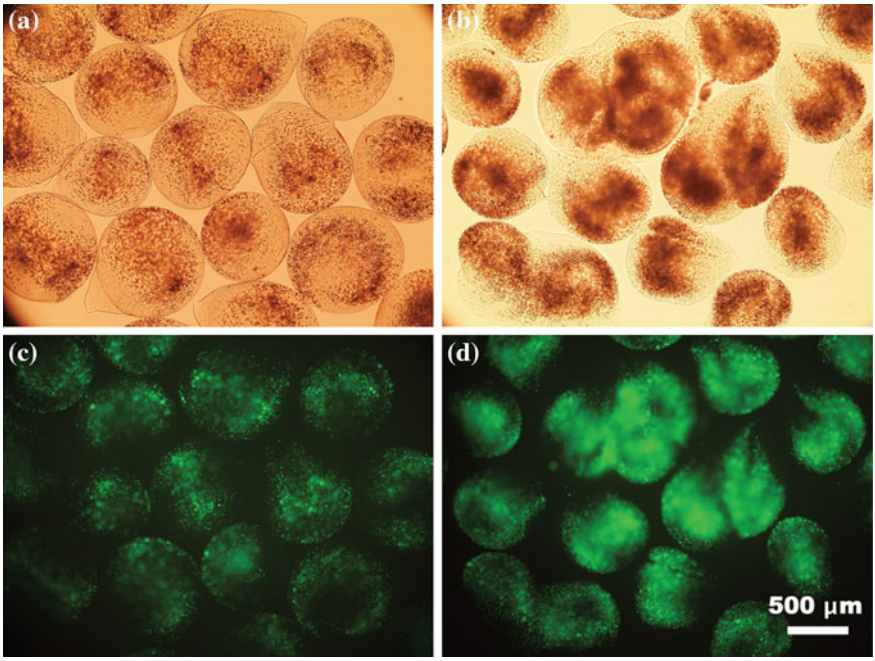

Fig. 4 Alginate Microbead visualization for optimization of cell density in beads. In pre-clinical studies, cells were encapsulated in 2% alginate beads. Encapsulated cells were engineered to express fluorescent signals (eGFP-green), which were used to determine cell count for optimization of cell density in beads. Images **a** and **c** (top row) show bright field, while images B and D (bottom row) show fluorescent images of the same beads. Beads on the left (image **a** and **b**) have a density of approximately 1.5×10^6 cells/ml alginate, while the beads on the right (image **c** and **c**) have a density of about 10×10^6 cells/ml alginate. The production of uniformly sized microcapsules is critical for the success of the microbeads in vivo

Other natural materials for cell encapsulation include *chitosan*, a polysaccharide derived from chitin. It has been used in application for encapsulations of drug delivery [82], and wound dressings [83]. In some cell encapsulations, a combination of alginate-chitosan (AC) was used for cell delivery applications [28, 84]. However, as with other alginate constructs, the encapsulation capsules have limited stability [85, 86]. Therefore, chitosan is more useful in combination with other materials, such as collagen, to form a layer with stronger mechanical properties, as needed in cell encapsulation devices [87].

Collagen, or its denatured form, *gelatin,* is a major protein component of the ECM (extracellular matrix) in tissues. It naturally provides support to tissues and is found in most building blocks of the body, like skin, cartilage, bones, blood vessels, and ligaments. In tissue engineering, collagen is used as a model scaffold due to its perfect biocompatibility, biodegradability, and the ability to promote cell binding [88]. Collagen can be obtained from animal tissues, is commercially available, and is used in many encapsulation applications for drug delivery [89].

Agarose is a polysaccharide derived from seaweed and used for mircroencapsulation of cells in microbeads or suspensions. However, a polysaccharide wall is not suited to keep the cells localized, since they can protrude through the matrix of the encapsulation layer after the formation of the capsules.

Suspensions of *cellulose sulfate*, originally derived from cotton, can be processed to form a semipermeable membrane around suspended cells. Both mammalian cells, as well as bacterial cells, have been shown to remain viable and continue to replicate within a cellulose sulfate capsule membrane [90]. Cellulose sulfate capsules filled with antibody-producing cells have been used for gene therapies and antibody treatments [25].

In the development of cellular macroencapsulation devices for humans, very few materials have been shown to be suitable for long-term implants. According to the FDA, alginate is the only approved material safe for use in humans [91]. Nevertheless, engineered synthetic polymers (PTFE) have been established as an encapsulation layer between implanted insulin-producing cells and host cells for macroencapsulation devices approved for clinical trials in humans. An example of a macroencapsulation device intended as an 'artificial pancreas' is further discussed in the pancreas section (part 5) of this book chapter.

4 Applications of Stem Cell Micro-encapsulation for Liver Disease

4.1 The Need for Liver Cell Therapies

The liver is the largest organ in the body, and the only one with the ability to regenerate from as little as 40% of its original mass. Rat livers, for example, can regenerate two thirds of their mass within a few weeks following acute injury [92]. Even though the liver is one of the organs best able to regenerate, it is also the organ most in demand for transplantation. As a blood filter, the liver is a target of toxic substances, including alcohol and viruses (hepatitis B/C) that compromise its function and ability to regenerate. Metabolic dysfunction, resulting in fat accumulation and aggravated fibrosis in the liver, is also destructive. Repeated adverse events in the liver ultimately result in damage and loss of function [93]. Such damage may also contribute to the development of liver cancer, which is on the rise globally [94]. More than 6000 liver transplants are performed every year. There are currently 17,000 people on the waiting list for a liver in America. The current mean wait time for a liver is 149 days for adults and 86 days for children, and these wait times are increasing. Every year, more than 1500 people die while waiting for a liver (American Liver Foundation, 2016). Therefore, there is a desperate need for livers. A therapy that would allow implantation of encapsulated stem cells to help restore liver function by replacing dysfunctional cells could ultimately mitigate this demand. Stem cell therapy could be used to keep the patient alive while they are

waiting for a liver transplant (so called 'bridging'). Advantages of cell micro-transplantation over using an entire organ include: more patients can be treated from a single donor organ; cells can be frozen and used later; surgery is less invasive; the procedure can be repeated, if needed, with low morbidity; and the procedure is less expensive [95].

4.2 Cells for Liver Therapeutics

Currently, primary hepatocytes do not survive or maintain their phenotype in vitro. Hepatocytes embedded in their natural microenvironment in vivo are long-lived and survive up to a year [96], but when removed from their microenvironment and placed in vitro, they lose their phenotype within a day, and perish, usually within a week [97]. Therefore, there is a need for stem cells to be used in liver cell therapy. Stem cells have several potential advantages over primary hepatocytes: they are self-replicating and capable of differentiating into other types of cells, by definition. Stem cells are capable of differentiating into the types of cells that may be required for liver regeneration.

Liver progenitor cells (LPC) have also been called oval cells, liver hepatic stem cells, atypical ductular cells, ductular hepatocytes, and intermediate hepatobililary cells [98]. LPCs are found in human, mice and rat livers [99]. LPCs are quiescent stem cells in the adult liver; they are activated to proliferate during liver injury, and can differentiate to both hepatocytes and cholangiocytes (also known as biliary epithelial cells, BEC), which line the bile ducts. LPCs are thought to be a heterogeneous population of cells in regard to their differentiation capacity and stage of differentiation [96]. These cells originate from the canals of Hering, the interface between hepatocytes and cholangiocytes. Their proliferation and differentiation are regulated by chemokines and cytokines produced by immune cells that are activated at an injury site [100–104]. Nonetheless, in the majority of cases, hepatocytes that assume proliferative capability, and NOT LPCs, are responsible for the bulk of liver regeneration [105, 106]. LPCs can become important during injury, but their role in this process is still not well understood. It is believed that LPCs become active when hepatocyte proliferation is impaired, but even this is being debated [106]. Recently, it was found that hepatocytes can be reprogrammed to LPCs in a mouse model of chronic liver injury [106] and that they can differentiate back to hepatocytes when transplanted into recipient, non-injured mice [107]. Their low frequency can make them difficult to isolate, thereby limiting the supply of LPCs. Additionally, there are concerns about carcinogenesis, because LPCs also occur in hepatocellular carcinoma [108].

Hepatoblasts are bipotent progenitor cells and can differentiate to hepatocytes or cholangiocytes similar to LPCs (see Fig. 1). They are isolated from human fetal livers during early gestation. Hepatoblasts proliferate well in vitro and are able to integrate and proliferate in injured livers of a host. However, due to their source, availability is extremely limited [105].

Non-liver specific stem cells, for possible use in liver therapy, include MSCs, ESCs and iPSCs (see Fig. 1). So far, MSCs, transplanted in a host with liver injury, do not, to any great extent, differentiate to functional hepatocytes (range 0.05–0.23% of implanted cells). To the contrary, the natural ability of MSCs to differentiate into myofibroblasts for tissue repair purposes at an injury site does increase unwanted liver fibrosis in vivo, and is particularly of concern in liver regenerative applications [109]. However, if implanted MSCs are encapsulated, differentiation input from the environment can be more rigorously controlled and stimuli towards fibrogenic differentiation could possibly be limited, leaving only beneficial paracrine effects of MSCs.

ESCs, on the other hand, have been differentiated to hepatocyte-like cells [110–112]. However this process is still limited in scale. Also, iPSCs have shown promise insofar as they differentiated to hepatocytes in vitro [40]. In summary, the identification of suitable cells to be used in encapsulated implantation devices for liver regeneration is still in need of further investigation.

4.3 Micro-encapsulation Designs

To find the best-suited encapsulation material specific for the needs of liver cells, alginate has been explored. Depending on the concentration, alginate is reported to have varying effects on liver cell differentiation. High concentrations maintain stemness and lower concentrations allow differentiation of liver cells [113]. The concentration most used in studies seems to be 2% (weight/volume). Combining alginate with other polymers, such as PLL and PEG, can strengthen the construct. Alginate, itself, is subject to degradation. Cho and Elazar found that viruses could infect encapsulated LPCs through cracks in the coating of alginate beads [114]. On the other hand, use of alginate for liver stem cell encapsulation can influence stem cell differentiation and improve function of mature hepatocytes. When ESCs were encapsulated in alginate microbeads and grown in vitro, the ESCs differentiated towards hepatocyte-like cells, as shown by Fang et al. Hepatocyte function was assessed by secretion of urea and albumin, glycogen storage, and cytochrome P450 transcription factor activity. A cell density of 5 million cells/ml in a 2% alginate concentration was determined to be optimal for stem cells to exhibit liver cell function. After about two weeks in vitro, ESCs differentiated into hepatocyte-like cells, as indicated by albumin secretion and positive staining for glycogen [59].

Some in vivo studies used MSCs for liver regeneration experiments. Meier et al. designed encapsulation material for microbeads that contained a combination of alginate and synthetic PEG for mechanical strength and stability. Beads were made in one step with interactions of calcium alginate molecules and covalent crosslinking from vinyl sulfone-terminated multi-arm PEG molecules [115, 116]. This material combination allowed adjustment of the permeability and swelling of the cell-carrying microbeads, making them more stable. In Meier's experiments, the new material was then injected in the peritoneal cavities of mice, with and without

encapsulated MSCs, to treat induced liver fibrosis. Experimental results showed that microcapsules loaded with MSCs had lower inflammatory effects on the mice compared to empty beads (negative control) or beads loaded with human fibroblasts. Furthermore, encapsulated MSCs had positive paracrine effects on the host by secreting interleukin-10 (Il-10), hepatocyte growth factor (HGF), vascular endothelial growth factor (VGF), insulin-like growth factor binding protein (IGFBP), and matrix metalloproteinase (MMP). The in vivo secreted proteins helped remodel the fibrotic tissue and decreased liver fibrosis overall. In comparison, in another study, injected MSCs WITHOUT encapsulation differentiated to collagen-producing fibroblasts after contact with host cells, which actually worsened the *chronic* liver problem by increasing fibrosis [117]. These two findings emphasize the fact that a microencapsulation device loaded with encapsulated cells can exhibit beneficial *paracrine effects*, where as, in the contrary, the same cells can contribute to unwanted fibrosis when not encapsulated. The results indicate that the avoidance of contact between host and implanted cells through an encapsulation layer is absolutely crucial to benefit the host in MSC derived liver regenerative therapies. A careful design of the encapsulation layer as a permeable, non-degradable casing for implanted cells, which will allow paracrine drug delivery in vivo, seems to be the key for implanted cellular drug delivery therapies, particularly for liver diseases.

4.4 Co-encapsulation

The last decade of research in tissue engineering has made it very apparent that for appropriate tissue regeneration, stem cells require two things: companion cells (e.g. stromal cells), and a 3-dimensional geometry of a cell culture growth environment. Cell encapsulation is inherently 3-dimensional and that has been found to improve hepatocyte-like function on its own [118].

The most investigated co-encapsulation for hepatic cellular therapy is MSCs and hepatocytes. Hepatocytes might be the best suited cells to treat acute liver disease, when there is no time for differentiation; while MSCs, with their paracrine ability to secrete beneficial proteins over a relatively long time period in vivo, might be good for treatment of chronic liver ailments. It makes sense that a combination of both, MSCs and hepatocytes encapsulated together, could provide an efficient long-term cellular therapy design for liver regeneration.

Liu and Chang designed in vivo experiments to co-encapsulate MSCs, derived from bone marrow, with primary rat hepatocytes. Microcapsules were loaded with both cell types and implanted in the peritoneum of Wistar rats [79]. Liu and Chang found that co-encapsulation largely increased hepatocyte viability. The ratio of co-encapsulated cells was 2:1 of hepatocytes to MSCs. To ensure immunoisolation by avoiding entrapment of cells in the surface of the microcapsule, a two-step encapsulation method, combining alginate and PLL material, was used. The length of cell survival in vivo and retrievability of viable co-cultures was assessed.

In comparison to prior observations, the enhanced encapsulation method resulted in a significant survival increase of the implants and successful retrieval of more than 90% of encapsulated co-cultures after 1 month of implantation. Furthermore, the two-step encapsulation method produced lasting microcapsules with therapeutically effective co-encapsulated cells eliminating most of the fibrosis and inflammatory responses in the host. Some vital hepatocyte clusters were retained in vivo for up to 3 months, indicating that this method could be useful for bridging patients waiting for a liver transplantation. However, in this study, function of encapsulated hepatocytes was not quantitatively assessed.

In another study, hepatocytes and MSC's were co-encapsulated 2:1 and implanted in the peritoneum of rats, in which *acute* liver failure had been chemically initiated [65]. The co-encapsulated cells dramatically improved survival rate and liver function in the treated rats. PLL-coated alginate was used to encapsulate the co-cultures, but the core of the microbeads was dissolved using sodium citrate. With this encapsulation design, it was demonstrated that MSCs were able to minimize inflammatory and fibrotic processes after acute liver failure through their paracrine effects, while hepatocytes could help replace lost liver tissue. MSC/hepatocyte treated rats had a 60% survival rate after one week, compared to 30% for rats treated with encapsulated hepatocytes only, and to 10% for untreated rats. Furthermore, less damage was done to the livers of rats treated with the co-encapsulated cells, as assessed by liver enzymes, plasma ammonia, and total bilirubin measurements. The improvement in survival rates in rats with acute liver injury indicates that treatment with co-encapsulated MSCs and hepatocytes holds potential for a cellular encapsulation therapy after acute liver damage.

4.5 The Future of Cellular Liver Regenerative Therapies

Stem cell encapsulation therapies for liver diseases hold great potential for both chronic and acute liver injuries. The use of stem cells can overcome disadvantages of hepatocytes, such as limited availability and inability to maintain proliferation and function in vitro. The microencapsulation of implanted stem cells for liver applications needs to be carefully designed, in order to direct differentiation to hepatocyte-like cells that benefit the host via paracrine effects while avoiding cell-to-cell contact with host cells. Co-culture of adult liver cells with stem cells provides a strong advantage for hepatocyte survival rate in vivo. Microencapsulated cellular implants have effectively increased survival rate after liver damage in rodent models. For *short-term* solutions, implants of encapsulated co-cultures of hepatocytes and MSCs carry the potential as a bridge therapy to liver transplantation. However many problems still need to be resolved before stem cell encapsulation for liver disease treatment is clinically feasible.

A major bottleneck in stem cell therapy is reproducibility. In vitro systems need to be controllable and scalable [119]. Protocols for stem cells must be robust and completely efficient. Cellular signaling must be better understood in order to enable

optimal differentiation of stem cells into functional hepatocytes and maintenance of functional hepatocytes in vivo. To date, stem cells have not been differentiated to fully functional hepatocytes, only hepatocyte-like cells [120]. Scale-up is still an issue. Also, differentiation requires expensive growth factors and other supplements. Synthetic compounds that will support these processes would be beneficial in regard to reducing variability and cost. Further development of sophisticated synthetic materials that solve the problems of biocompatibility, localization, and differentiation of stem cells will be a major step in the advancement of therapeutic stem cell encapsulation.

5 Application of Stem Cell Macro-encapsulation for Diabetes Mellitus

5.1 The Need for Islet Cell Therapies

In 2012, more than 371 million people had diabetes, and it is predicted that by 2030, this number will increase to 552 million people worldwide (International Diabetes Foundation, 2016). Type I diabetics, and about 25% of Type II diabetics, rely on exogenous insulin for survival [121]. In the past 10 years, insulin-dependent diabetes cases have been successfully treated by transplanting donor *Islet of Langerhans* cells in the approach to replace endocrine tissue. However, with only 10,000 organ donors per year, the major obstacle of islet transplantation is the limited human donor supply [122]. Furthermore, in order to avoid transplant rejection, immune responses against the foreign islet tissue in the recipient need to be suppressed. Life-long administration of immunosuppressant medications are known to have harmful side effects and favor the manifestation of other unrelated infections [123]. Together these limitations justify the search for alternative sources of insulin-producing cells. In xenotransplantation approaches, swine islet cells were used for implantation, since swine pancreases were readily available, and the human and pig insulin differ only by one amino acid [53]. However, even with immunosuppressant medications, the swine cells were rejected by both humoral and cellular immune reactions, due to immunologic species barriers [124].

5.2 Islet Cell Encapsulation

In search of immunoprotective methods for islet cell implantation, sophisticated encapsulation techniques for cells are explored as an alternative to immunosuppressants. Initially, therapeutic approaches included encapsulation of small numbers of individual islets coated by semipermeable membranes that were intended to prevent contact between implanted cells and host cells [16]. In subsequent rodent and primate experiments, some microencapsulation approaches were successful in

increasing cell survival in vivo [125, 126]. Early-phase human trials with mature porcine islets, embedded in alginate microcapsules and injected intraperitoneally in diabetic patients, were conducted by a New Zealand company named 'Living Cell Technologies' [127]. Despite initial promising achievements in the field of encapsulated islet transplantation, pericapsular fibrosis led to increased failure rates of the implanted microcapsules [128]. Several studies confirmed that the choice of encapsulation material is responsible for the activation of foreign body responses in the host, as certain compositions of alginate stimulates monocytes and macrophages to produce pro-inflammatory cytokines [81, 129, 130]. The development of more refined encapsulation material was crucial to further advance encapsulated islet cell implantation technology [78, 131–133]. Barium crosslinking methods of alginate materials, for example, have been demonstrated to reduce antibody- or cytokine-mediated islet-injury in vivo [19]. Other in vivo experiments revealed complications with the injection site of the microcapsules [123]. For example, APA microcapsules were loaded with 1–3 islets and injected into the liver of diabetic patients via the portal vein [134]. However, the injection of the microcapsules into the portal vein led to complications, such as hypertension and portal thrombosis [135]. Alternative injection sites, like the peritoneum, the kidney capsule, and subcutaneous tissues, were tested in mice and primates [136–138]. Generally, with advances in encapsulation materials, immune and foreign body responses by the host have been reduced, in comparison to un-encapsulated islet cells. However, even encapsulated islets have not been proven viable in vivo for long periods of time. Due to the porosity of the cell-enclosing microcapsules, missing microenvironmental factors, and the choice of the implantation site, only short-term effects of mature islet cell implantations were reported to be successful [139, 140]. Without sufficient survival rates of the implanted islets, the *micro*encapsulation approach of single islets is not ready to be suitable for long-term application in humans.

To provide durability and retrievability of transplanted islet cells as well prevent cell escape, approaches with a larger encapsulation chamber led to the first *macro*encapsulation device with living pancreas islet cells [141]. In order to supply the larger number of implanted cells inside the encapsulation chamber with nutrients, diffusion distances to blood vessels in close proximity of the extravascular device surface need to be kept minimal. Thus, the original designs of a macroencapsulation device were focused on cellular monolayers, encapsulated in a flat-sheet double membrane chamber system that incorporated 2-dimensional monolayers of cells in a planar islet sheet device [142]. Only later, it was established that a 3-dimensional growth chamber does encourage cell growth and survival, as it better mimics the cell's natural environment [143]. Furthermore, the initial choices of encapsulation material (nitrocellulose, crude alginate, acrylonitrile, and agarose) with little biocompatibility or initiators of inflammatory side effects made the early macroencapsulation device less successful. Toxicity of the encapsulation layer and other factors, e.g. device surface irregularities, triggered fibroblast attachment and activated nonspecific foreign body responses, resulting in subsequent necrosis of the encapsulated tissue and device failure [144]. When *adult* islet cells were

co-encapsulated with mesenchymal *stem cells* (*MSCs*), fibrotic growth could be limited due to positive anti-fibrotic paracrine effects of encapsulated MSCs, and the macroencapsulation devices exhibited prolonged survival rates in vivo [145, 146].

5.3 Stem Cells for Treatment of Diabetes Mellitus

A more recent development is moving away from the implantation of *mature* islet cells with limited life span. The new focus is on the use of *stem cells* in macroencapsulation devices. However, before macroencapsulated stem cell devices can become a possibility for treatment of diabetic patients, stem cell treatment protocols are in need of being refined and standardized, so that the differentiation of human ESCs or iPSCs into insulin-producing islet-like cells in vitro and in vivo can be repeatedly documented. The pioneering discovery was that human primary pancreatic islet progenitor cells, derived from human embryonic stem cells, will continue to differentiate in vivo into insulin-producing cells inside the encapsulation chamber. Studies showed that glucose-stimulated insulin responses started to increase after a few weeks post-implantation, suggesting that implanted encapsulated primary islet progenitor cells had matured to insulin-producing cells in vivo [147]. In some instances, however, mass transport in and out of the implanted device chamber was compromised by wound healing and fibrotic host reaction processes, i.e. the foreign body response, leading to the shut-down of paracrine communication between cells, and ultimately resulting in insufficient insulin secretion [148]. The device encapsulation layer's chemical and mechanical cues seemed to stimulate fibrotic processes in the host due to its biocompatibility, its architecture, and the oxygen permeability of the material itself [149]. Also, it has been demonstrated that the stiffness of the alginate substrate can affect stem cell differentiation by triggering transcriptional responses [150]. Direct effects of the encasing material on surrounding tissues and implanted cells show the significance of the need to carefully choose the design and material for an encapsulation device, in order to ensure long-term implant survival and to achieve intended differentiation of cells in vivo.

In summary, two major obstacles of encapsulated stem cell transplantation, intended for in vivo insulin production devices, need to be addressed: (1) the stimulation of hESCs or iPSCs to differentiate into insulin-producing cells in vivo, while the inherent ability of hESC/iPSCs to proliferate rapidly in an unpredictable manner is blocked, and (2) the risk of graft failure due to immune response attacks by the host or fibrotic reactions resulting from foreign body and wound repair processes [19]. To provide a successful macroencapsulation device for the treatment of diabetes, device engineering tools have to be optimized to provide a biocompatible, immunoprotective, semi-permeable growth chamber with ideal conditions for stem differentiation in a foreign host, while promoting host vascularization for nutrient exchange and insulin distribution.

5.4 Stem Cell Differentiation

Stem cell engineering refers to the ability to manipulate and control the powerful potential of stem cells to self-renew and/or to differentiate into specialized cells. Particularly, in the manipulation of human stem cells to specialize into insulin-secreting pancreatic cells in long-term implantation devices, detailed sequential steps in the differentiation pathway have to be identified, identically repeated, standardized, controlled, and they have to provide the ability to be scaled–up for manufacturing purposes [151]. The process has to be efficient and homogenous to ensure repeatable functionality of the implantable cells. This can be achieved with standardized protocols that promote precise sequential changes in the cellular microenvironment in vitro and in vivo. Stem cells need to be trained to respond to signals (glucose) and secrete proteins (insulin) required for glucose control. This can be executed by means of specialized cell media with added growth and other factors in vitro and/or via ECM or cell-cell signals around implanted cells in vivo. In order to allow for the desired differentiation steps to occur at specific time points, in vitro and in vivo steps need to be optimized with precision. In 2014 and 2015, three independent research groups developed refined stem cell treatment protocols to create insulin-secreting cells [152–154]. The commonalities of the protocols were used to optimize the cell differentiation protocol [155] for use in cellular macroencapsulation devices, which potentially could function as an 'artificial pancreas' device.

Possible stem cells used in macroencapsulation devices for treatment of diabetes are iPSCs and human ESCs (hESC) (see Fig. 2). IPSCs, originated from human fibroblasts, have shown the ability to differentiate into functional β-cells in vitro, which responded to glucose stimulation [156]. To generate iPSCs, reprogramming of the adult fibroblasts was achieved by the activation of three transcription factors (OCT4, SOX2, KLF4) [34, 156, 157]. Also, adult pancreatic ductal or epithelial cells, and even α-cells, have been genetically reprogrammed to iPSCs using adenoviral vectors and certain combinations of transcription factors [158]. After iPSCs are successfully engineered, the cells are then initiated to differentiate into β-cell-like cells that respond with insulin production upon a glucose challenge [159]. Although approaches to use iPSCs for the treatment of insulin-dependent diabetes are promising, clinical use is not yet feasible due to targeting of reprogramming transcription factors and viral mutagenicity [160]. Non-viral mediated introduction of reprogramming factors however, could enable safe clinical use.

ESCs can be directed to differentiate into functional endocrine pancreatic cells without the use of viral vectors, but instead by means of reproducible differentiation protocols, additions to growth media, selected hydrogels, and co-culturing approaches [161]. However, large populations of original obtained endocrine cells from ESC differentiation were poly-hormonal, meaning single cells are expressing simultaneously somatostatin, glucagon, and insulin. Cells expressing multiple hormone markers within a single cell are found to have poor responses to a glucose challenge in terms of insulin release [160]. Instrumental changes in the

development of a protocol that could differentiate hESCs into islet-like cell populations containing single-hormone-producing, insulin-secreting pancreatic endoderm cells, were based on studies of D'Amour et al. [162] and Kroon et al. [163]. The differentiation protocols were subsequently optimized for in vivo implantation by Agulnick et al. in 2015 [155]. All three research groups used a 2-step procedure with differentiation of hESCs to pancreatic progenitor cells (PPC) in vitro, and implantation of the encapsulated PPCs for maturation into islet-like cells in vivo (see Fig. 2). In this protocol, hESCs are initiated on a desired pathway to differentiate into committed pancreatic progenitor cells (PPC) in vitro with 7 distinct steps in the use of growth media, containing Activin A and Wnt3A at different time points. With these steps, complex signaling pathways are suppressed or promoted during the initiation of the differentiation process. Wnt signaling has been shown to promote proliferation of PPCs in the developing mouse, and Activin family signaling has been implicated in pancreatic specification [155]. Unique to this treatment protocol is the combination of adherent and suspension culturing during the in vitro differentiation step. 15 days of adherent cell culturing in flasks is followed by a 1-day aggregation step that is finalized by 12 days of cell differentiation in suspension, allowing 3-dimensional growth of cell clusters. HESCs develop into pancreatic endoderm cells (PPCs) during this step (see Fig. 2). After the differentiation to PPCs in suspension is completed, a 3-day preparation step, using various suspension formulations, prepares the cells for the implantation device. The implant preparation step enables progress to final maturation of the implanted cells, which does take place in vivo, post implantation, and produces insulin-producing islet-like cells inside the implant chamber [151]. Resulting cell populations from hESCs treated with this differentiation protocol exhibited about 80% of matured cells in the implant with endoderm characteristics and many expressed insulin [155, 163]. Main factors required to generate glucose-responsive, insulin-producing cells were culturing in suspension (3D culture), and the temporary activation or deactivation of certain signaling pathways during cell differentiation via substances in the microenvironment of the hHSCs and PPCs [152, 154]. Specifically, the presence of retinoic acid to dampen the sonic hedgehog signaling pathway (a known inhibitor of pancreas development) [164], and the presence of the thyroid hormone tri-iodothyronine (T3), known to be required for liver development [165], were demonstrated to be influential in the cell differentiation steps. It was also found that high retinoic acid levels during the beginning of the hESC differentiation, followed by treatment with specific growth factors (EGF/KGF), reduced the number of poly-hormonal cells [154].

Furthermore, procedures to preserve and store the implantable cells were established, which is necessary to generate a consistent and robust population of implantable cells suitable for cellular therapeutic manufacturing. Experiments to cryopreserve and re-aggregate treated hESCs showed no significant difference in functionality and insulin secretion after implantation in vivo [166].

Itkin-Ansari et al. demonstrated that encapsulated hESC derived islet-PCs acquire glucose-responsiveness in vivo without a significant change in biomass or any evidence of cell escape from the encapsulation chamber [121]. In her

experiments, macroencapsulated cellular devices were implanted in mice, and located and imaged with bioluminescent imaging techniques in vivo (Fig. 5). In immunohistochemical images of explanted devices (Fig. 6a), encapsulated matured islet-like cells are visible inside an implantation device (device encapsulation membrane layers appear red due to autofluorescence), and did not reveal an increase in cellular biomass over time. This finding may indicate that the matured PPCs lost the proliferation and self-renewal capacity of hESCs. The finding of constant cellular biomass in a cellular implant device eliminates the general concern with stem cells to uncontrollably self-renew in vivo, possibly leading to overpopulation of implanted stem cells. Furthermore, as visible from immunohistochemistry images of explanted devices (Fig. 6b), the encapsulated cells show a high percentage of insulin-expressing cells (green), as well as a minor amount of glucagon-expressing cells (red). It was noted that no single cells in the graft were positive for both hormones (see Fig. 6b). These experiments showed that hESC derived, implanted, and matured endocrine cells either expressed insulin or glucagon, but not both, confirming that progenitor cells in the device had matured along the desired distinct endocrine lineages [121].

The combination of several recent advances in diabetes-related stem cell research may finally bring bench-top research to the clinic, and reinstates hope to manufacture a therapeutic encapsulation stem cell device, adequate to treat diabetic

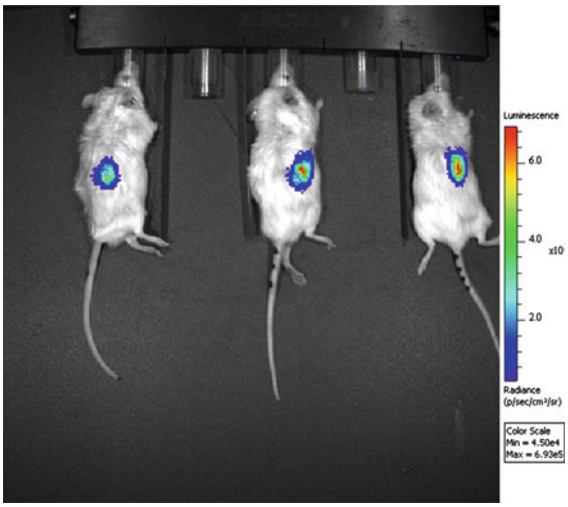

Fig. 5 Whole mice imaging with bioluminescent cellular device implants. *First published by Elsevier* [121]. Mice were implanted with macro-encapsulation devices containing pancreatic progenitor cells transducted with a self-inactivating lentivirus carrying firefly luciferase for visualization. Live, anesthetized, implant-carrying mice were scanned in vivo using an IVIS Spectrum imaging platform (Perkin-Elmer) to quantify luciferase expression of implanted cells. No significant change in biomass or any evidence of cell escape from the encapsulation chamber was detected

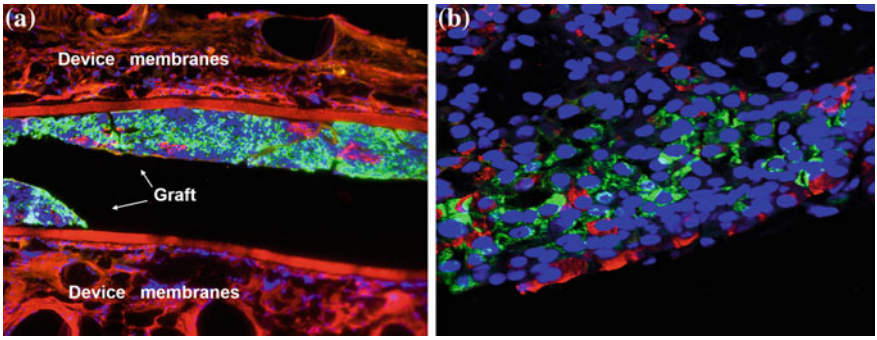

Fig. 6 Immunohistochemical images of a cell graft from an explanted encapsulation chamber in mice experiments. *First published by Elsevier* [121]. Cross-sections through an encapsulated cell graft show matured hormone-producing islet-like cells in vivo. **a**: Device cross-section. A vital cell graft is visible inside the encapsulation chamber (device membrane appears red due to autofluorescence). Vital graft cells are immunostained for insulin (green) and glucagon (red). Cell nuclei appear blue. Human ESC-derived pancreatic cells, encapsulated in the device, secrete human insulin (green) in vivo. Magnification: 100x. **b**: High power view of cellular graft. The cell graft is compromised of a high percentage of insulin positive cells (green) and a smaller amount of glucagon positive cells (red). Cell nuclei appear blue. It was noted that no single cell in the graft was positive for both hormones, confirming that hESC-derived pancreas progenitor cells had matured into distinct endocrine single-hormone-producing pancreatic cells inside the encapsulation device in vivo. Magnification: (1200x)

humans. In these recent experiments, optimized hESC growth and differentiation protocols were used to successfully obtain implantable islet-like cell clusters.

In summary, milestones have been met to enable hESCs to form endoderm cells with subsequent development through pancreatic PCs into mature endocrine cells, capable of synthesizing pancreatic hormones in vivo. Recent refinement in the treatment protocols of hESCs has generated a consistent, transplantable cell population that differentiates in vivo, producing insulin-secreting cells. Furthermore, bioprocessing strategies to enable controlled manufacturing of these cells, have opened the path for possible clinical use in humans.

5.5 Macroencapsulation-Device Engineering

Maintaining long-term graft viability, while eliminating the need for immunosuppressive medication, can only be accomplished through encapsulation [167]. The biomaterial used for the encapsulation layers of the device has to provide specific properties that resonate with the implanted cells, as well as protect them from the host, yet the layer must allow transportation of paracrine and endocrine products. Biomaterial research and chemical advances provide continuous opportunities to design, synthesize, test, and optimize desired polymers for encapsulation purposes [168]. Modern encapsulation device engineering for clinical applications in humans

includes the testing of biocompatible materials, risk management, concept and feasibility assessment, design controls, and process validation controls. For clinical purposes, the device also needs to be accessible for monitoring with common clinical imaging systems and allow for easy retrieval. With the advancement of sophisticated micro- and nano-manufacturing techniques, it has become possible to 'engineer' a membrane for encapsulation of live cellular grafts with precise morphologies, surface characteristics, and immunoisolation properties [169]. In recent experiments, a synthesized polymer PTFE (PolyTetraFluoroEthylene) was coated with alginate and engineered for an encapsulation device to be implanted under the skin of humans [169]. PTFE has been shown to limit the induction of fibrotic repair processes and to exhibit good vascularization-stimulating qualities, allowing for better cell viability in implants [170, 171]. An important factor for these qualities is the pore size of the engineered PTFE. Smaller pore sizes in the sub-micron range have been demonstrated to provide good immunoprotection for transplanted cells in vivo [121, 172]. Immunological cells, such as macrophages and leukocytes, about 6–10 microns in diameter, cannot pass through pores sized in the sub-micron scale. On the other hand, larger pore sizes in the host-interface layer of PTFE material, starting at about 5 μm, initiated a substantial increase in vascularization on the membrane-tissue surface. This observation remained, even when the larger pore size membrane was laminated to an inner smaller pore size membrane to prevent cell protrusion [169]. Silicon micro-machining allowed the production of the macroencapsulation material with uniform and well-controlled pore sizes, channel lengths, and surface properties [169, 173].

Based on the ability to engineer precise pore sizes in synthetic polymers and the consequential tissue reactions that are triggered by different pore sizes, an American company named 'Theracyte' developed an advanced material for a durable, planar macroencapsulation device. The innovation was an encapsulation membrane consisting of a laminated PTFE bilayer with 2 different pore sizes. The new design of the encapsulation membrane combined a PTFE outer layer with larger pore size (5 μm) and a PTFE inner layer with sub-micron pore size (0.45 μm) [169]. This specialized PTFE material was another step toward an implantation prototype device, combining angiogenic stimulation properties, initiated by larger pores at the surface with host cells, and crucial immunoisolation properties, provided by the smaller pores of the inside layer. Immunoisolation characteristics could be further enhanced by other nano-techniques, such as precise surface alignment of nano-fibers and coating with peptide-modified alginate hydrogels that inhibit inflammatory cell surface receptors [173]. The Theracyte encapsulation device was unbreakable, retrievable, supported neovascularization near the implant, kept implanted cells contained, and avoided host cell contact via the small pore inner membrane layer. In subsequent studies, this device was used for implantation of mature islet cells in pre-clinical rodent experiments [174–176]. One research group reported survival of cell implants in vivo in rats for up to 6 months using the Theracyte macroencapsulation device [177]. However, human clinical trials using mature islet cells, macroencapsulated within the Theracyte device, remained unsuccessful for long-term implantation due to fibrotic host reactions around the

implant. Fibroblast attachment on the polyester mesh that was attached to the outer 5 μm encapsulation layer of the device hindered long-term device performance in vivo [170].

The development of new multi-layered encapsulation material becomes even more crucial for implantation of stem cells. Synthetic membranes of macroencapsulation devices for stem cells do not only need to provide immunobarrier properties (see Fig. 3) and neovascularization stimuli, but also have to ensure an environment for differentiation and maturation of implanted stem cells within the encapsulation chamber. The design and material of devices like the Theracyte one, holds great potential for the implantation of stem cells, since oxygenation and effective nutrient and waste exchange through enhanced angiogenesis around the implant was achieved. Neovascularization with ample blood supply to the implanted stem cell mass is absolutely crucial for survival of the implant [149]. Once stem cells mature to insulin-producing endocrine cells in vivo, the distribution of secreted therapeutic substances (e.g. insulin) is also ensured by proximity to the host's vasculature. Furthermore, desired insulin secretion of the implanted cells can be regulated by the host's blood glucose levels, as initiated through paracrine communication from close-by blood vessels through the device encapsulation layer [169].

A biotechnology company in California USA, 'ViaCyte LLC', has pioneered in the area of encapsulated stem cell transplants. ViaCyte has demonstrated the feasibility of encapsulated hESCs using a Theracyte-like device. In Fig. 7, an example of neovascularization around the macroencapsulation device from clinical studies is shown. The cellular implant device is carrying insulin-secreting islet-like cells, which were derived from hESCs, differentiated into islet progenitors prior to implantation, and which matured in vivo. The device was explanted after 18 weeks in vivo and shows sufficient neovascularization on the implant's surface (see Fig. 7).

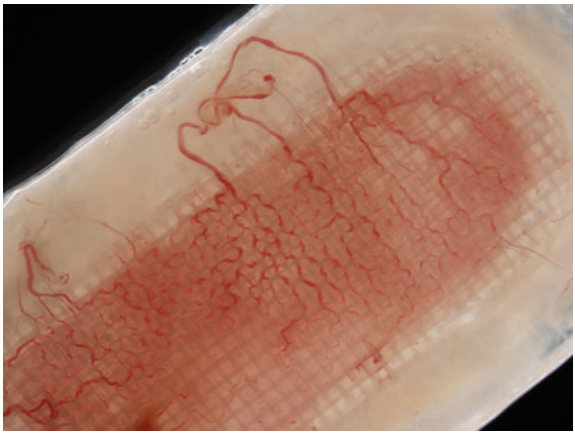

Fig. 7 Neovascularization around a micro-encapsulation device for treatment of Diabetes Mellitus. *Image provided by ViaCyte.* The micro-encapsulation device becomes densely vascularized by the host after 18 weeks of implantation. Vascularization is critical for robust engraftment and function of a cell-carrying device

5.6 The Synergy Between Stem Cell Therapy and Device Engineering

As a true Bioengineering approach, combining expertise across disciplines, ranging from nano-engineering, biomaterials, biotechnology, and tissue engineering, into the fields of immunology and clinical medicine, we can begin to address the multiple challenges that are involved in translating encapsulated stem cell therapy from the laboratory to the clinic. Recent improvements in cellular graft viability, due to encapsulation methods with improved biomaterial manufacturing and purification techniques, as well as the identification of safe, reliable, scalable tissue sources with refinement in stem cell isolation and culturing techniques, may make it possible to introduce a successful cellular implantation device into the clinic [19].

With the hope of providing prolonged periods of insulin independence for diabetic patients, recent research advances have utilized many modern technologies to develop a cellular macroencapsulation device, using un-differentiated human ESCs to replace dysfunctional islet cells in diabetic patients. It has been shown that encapsulated hESCs can be successfully directed down an endocrine lineage, implanted without direct contact to the host environment, and that the hESC derived differentiated cells can deliver optimal insulin production after 3–5 months of maturation in vivo, as determined by improved glucose-stimulated insulin responses and C-peptide levels [163]. This finding, as similar to previous findings with primary human islet progenitor cells, suggests that stem cell differentiation continued after encapsulation and reached its optimum performance in insulin delivery after 3–5 months of implantation [147]. The example of the San Diego Biotechnology company ViaCyte demonstrates how stem cell therapies and device engineering are utilized to develop an implantable stem cell macroencapsulation device that shows promise as an in vivo cellular therapeutic tool to treat Diabetes Mellitus diseases. In Fig. 8, a histological image of a cross-section through a ViaCyte encapsulation device, after 18 weeks of implantation in a clinical trial, is shown. In the cross-section through the device chamber, encapsulated islet-like cells show viability (see Fig. 8a H&E histological staining) and development of insulin-producing cells in vivo (see Fig. 8b immunohistochemical stain for insulin, insulin appears red).

Starting with the original idea of implanting stem cells in a patient that then differentiate into therapeutic cells and deliver insulin in vivo, many challenges have been successfully addressed with bioengineering techniques and an engineered encapsulation device that provides immunoisolation from host cells, while allowing paracrine information, as well as nutrients, to pass. Successful preclinical results with a stem cell implantation device that could produce and deliver insulin in vivo, have lead to clinical trials. ViaCyte is currently carrying out a 3-year, human phase I/II clinical trial to assess safety and efficacy of the implant system, using stem cell-derived cell sources for an encapsulated cell replacement therapy in Diabetes

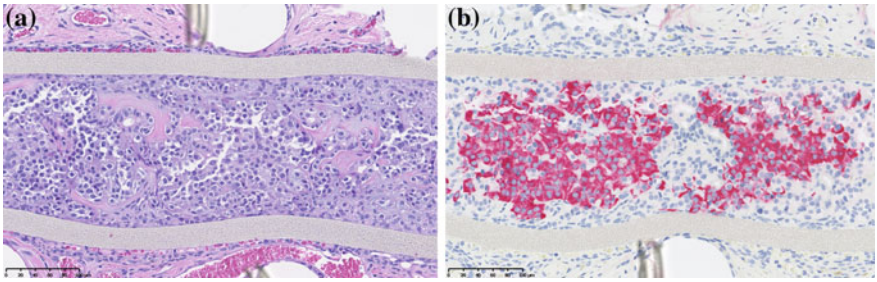

Fig. 8 Cross-section through an encapsulation implant. *Image provided by ViaCyte.* Histological images of cross-sections through a cell-carrying encapsulation device show vital, hormone-producing cells inside the encapsulation chamber (gray layers on top and bottom) after 18 weeks of implantation in clinical trials. **a**: H&E stain. Implanted encapsulated multipotent pancreatic progenitor cells matured into vital hormone-producing pancreatic cell types in vivo. **b**: Immunohistochemical stain for insulin (red) illustrates the differentiation into functional insulin-producing pancreatic cells

Mellitus [178]. Human subjects are Type I Diabetes Mellitus patients. The device is implanted subcutaneous and can be easily removed. Looking into the future, ViaCyte is also developing a platform for cryopreserving undifferentiated hESCs to provide the necessary foundation for scale-up and manufacturing needs of the cellular implantation device, if clinical trials prove to be successful in long-term settings.

In summary, many challenges were faced to develop an encapsulated stem cell device to treat Diabetes Mellitus. Issues like host vascularization and immune rejection of the implant seem to have been successfully addressed. However, long-term stability of cellular implants seems to be the bottleneck. Even if initial wound healing host reaction to an implant is successfully suppressed, long-term host tissue reconstruction via tissue repair mechanisms may trigger scar tissue and fibrotic host responses around the implant over time, which could negatively influence the long-term implant performance. To create a therapeutic cellular implant device that continuously provides insulin-independence for diabetic patients, long-term interface reactions between host and implant still need to be further investigated. Nevertheless, in the clinic, a cellular implantation device using encapsulated stem cell therapy for insulin-dependent Diabetes Mellitus patients has never been closer.

6 Conclusion

The synergy of stem cell therapy and medical device engineering for liver disease and Diabetes Mellitus has led to great progress in the development of encapsulation techniques with stem cells for human implantation therapies. The first clinical trials are underway. However, diabetic human subjects will not be able to benefit from

these achievements immediately, as reproducible results from multi-centered, multidisciplinary, randomized, controlled clinical trials will be required to establish treatment modalities for patients with diabetes. Nevertheless, the hope for life-quality improving treatments and the elimination of the need for daily insulin injections is a driving force in research and clinical therapy. The life-threatening shortage of human donor livers and pancreases is rationale to push forward with stem cells therapies and micro-and macroencapsulation techniques for therapeutic applications in humans. The idea of a bio-artificial pancreas or a cell-based liver replacement therapy has gained strong interest in recent research and therapy through encapsulation devices. Due to new bioengineering advancements and the better understanding of stem cell biology, the synergy between stem cell therapy and device engineering may provide promising options for new treatments of human disease in the future.

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References

1. Everhart, J. E., & Ruhl, C. E. (2009). Burden of digestive diseases in the United States part III: Liver, biliary tract, and pancreas. *Gastroenterology, 136*(4), 1134–1144.
2. Yadav, D., & Lowenfels, A. B. (2013). The epidemiology of pancreatitis and pancreatic cancer. *Gastroenterology, 144*(6), 1252–1261.
3. Congress, I. L. (2016). Fast facts about liver disease—The international Liver Congress 2016, April 13–17. The Home of Hepatology. http://2016.ilc-congress.eu/wp-content/uploads/2016/04/Liver-disease-backgrounder.pdf.
4. Blachier, M., Leleu, H., Peck-Radosavljevic, M., Valla, D. C., & Roudot-Thoraval, F. (2013). The burden of liver disease in Europe: A review of available epidemiological data. *Journal of hepatology*, *58*(3), 593–608.
5. Lévy, P., Domínguez-Muñoz, E., Imrie, C., Löhr, M., & Maisonneuve, P. (2014) Epidemiology of chronic pancreatitis: burden of the disease and consequences. *United European Gastroenterology Journal, 2*(5), 345–354.
6. Peery, A. F., Crockett, S. D., Barritt, A. S., Dellon, E. S., Eluri, S., Gangarosa, L. M., … Schmidt, M. (2015). Burden of Gastrointestinal, Liver, and Pancreatic Diseases in the United States. *Gastroenterology, 149*(7), 1731–1741 e3.
7. Zaret, K. S. (2008). Genetic programming of liver and pancreas progenitors: Lessons for stem-cell differentiation. *Nature Reviews Genetics, 9*(5), 329–340.
8. Rous, P., & Murphy, J. B. (1912). The histological signs of resistance to a transmissible sarcoma of the fowl. *Journal of Experimental Medicine, 15*(3), 270–286.
9. Bisceglie, V. (1933). Uber die antineoplastische Immunitat: (I) Heterologe Einpflanzung von Tumoren in Huhnerembryonen. *Zeitschrift für Krebsforschung, 40,* 122–140.
10. Bisceglie, V. (1933). Uber die antineoplastische Immunität; (II) Die Wachstumfahigkeit von heterologen Geschwulsten in erwachsenen Tieren nach Einpflanzung in Kollodiumsackchen. *Zeitschrift für Krebsforschung, 40,* 141–158.
11. Chang, T. M. (1964). Semipermeable microcapsules. *Science, 146*(3643), 524–525.

12. Federlin, K., & Raptis, S. (2001). Ernst F. Pfeiffer: His impact on diabetes research and treatment over the last 45 years. *Experimental and Clinical Endocrinology & Diabetes, 109* (2), S83–S85.
13. Bowen, K. M., Andrus, L., & Lafferty, K. J. (1980). Successful allotransplantation of mouse pancreatic islets to nonimmunosuppressed recipients. *Diabetes, 29*(1), 98–104.
14. Lafferty, K. J., Andrus, L., & Prowse, S. J. (1980). Role of lymphokine and antigen in the control of specific T cell responses. *Immunological Reviews, 51,* 279–314.
15. Chick, W. L., Perna, J. J., Lauris, V., Low, D., Galletti, P. M., Panol, G., et al. (1977). Artificial pancreas using living beta cells: Effects on glucose homeostasis in diabetic rats. *Science, 197*(4305), 4780–4782.
16. Lim, F., & Sun, A. M. (1980). Microencapsulated islets as bioartificial endocrine pancreas. *Science, 210*(4472), 908–910.
17. Cima, L. G., Vacanti, J. P., Vacanti, C., Ingber, D., Mooney, D., & Langer, R. (1991). Tissue engineering by cell transplantation using degradable polymer substrates. *Journal of Biomechanical Engineering, 113*(2), 143–151.
18. https://ki.mit.edu/people/faculty/langer. *Robert S. Langer*. 2017.
19. Krishnan, R., Alexander, M., Robles, L., Foster III, C. E., & Lakey, J. R. (2014). Islet and stem cell encapsulation for clinical transplantation. *The Review of Diabetic Studies*, *11*(1), 84–101.
20. Soon-Shiong, P., Heintz, R. E., Merideth, N., Yao, Q. X., Yao, Z., Zheng, T. I. A. N. L. I., … Mendez, R. (1994). Insulin independence in a type 1 diabetic patient after encapsulated islet transplantation. *Lancet, 343*(8903), 950–951.
21. Calafiore, R., Basta, G., Luca, G., Lemmi, A., Montanucci, M. P., Calabrese, G., … Brunetti, P. (2006). Microencapsulated pancreatic islet allografts into nonimmunosuppressed patients with type 1 diabetes: first two cases. *Diabetes Care, 29*(1), 137–138.
22. Löhr, M., Bago, Z. T., Bergmeister, H., Ceijna, M., Freund, M., Gelbmann, W., … Henninger, W. (1999). Cell therapy using microencapsulated 293 cells transfected with a gene construct expressing CYP2B1, an ifosfamide converting enzyme, instilled intra-arterially in patients with advanced-stage pancreatic carcinoma: a phase I/II study. *Journal of Molecular Medicine, 77*(4), 393–398.
23. Löhr, M., Hoffmeyer, A., Kröger, J. C., Freund, M., Hain, J., Holle, A., … Nizze, H. (2001). Microencapsulated cell-mediated treatment of inoperable pancreatic carcinoma. *Lancet, 357* (9268), 1591–1592.
24. Löhr, M., Kroger, J. C., Hoffmeyer, A., Freund, M., Hain, J., Holle, A., … Saller, R. (2003). Safety, feasibility and clinical benefit of localized chemotherapy using microencapsulated cells for inoperable pancreatic carcinoma in a phase I/II trial. *Cancer Therapy*, *1,* 121–131.
25. Pelegrin, M., Marin, M., Noel, D., Del Rio, M., Saller, R., Stange, J., … Piechaczyk, M. (1998). Systemic long-term delivery of antibodies in immunocompetent animals using cellulose sulphate capsules containing antibody-producing cells. *Gene Therapy*, *5*(6), 828–834.
26. Hartgerink, J. D., Beniash, E., & Stupp, S. I. (2001). Self-assembly and mineralization of peptide-amphiphile nanofibers. *Science, 294*(5547), 1684–1688.
27. Kobayashi, T., Aomatsu, Y., Iwata, H., Kin, T., Kanehiro, H., Hisanaga, M., … Nakajima, Y. (2003). Indefinite islet protection from autoimmune destruction in nonobese diabetic mice by agarose microencapsulation without immunosuppression. *Transplantation, 75*(5), 619–625.
28. Haque, T., Chen, H., Ouyang, W., Martoni, C., Lawuyi, B., Urbanska, A. M., & Prakash, S. (2005). In vitro study of alginate-chitosan microcapsules: an alternative to liver cell transplants for the treatment of liver failure. *Biotechnology Letters, 27*(5), 317–322.
29. Young, S., Wong, M., Tabata, Y., & Mikos, A. G. (2005). Gelatin as a delivery vehicle for the controlled release of bioactive molecules. *Journal of Controlled Release*, *109*(1–3), 256–274.
30. Dong, H., Fahmy, T. M., Metcalfe, S. M., Morton, S. L., Dong, X., Inverardi, L., … Wang, H. (2012). Immuno-isolation of pancreatic islet allografts using pegylated nanotherapy leads

to long-term normoglycemia in full MHC mismatch recipient mice. *PLoS One*, *7*(12), e50265.
31. Kim, A. R., Hwang, J. H., Kim, H. M., Kim, H. N., Song, J. E., Yang, Y. I., … Khang, G. (2013). Reduction of inflammatory reaction in the use of purified alginate microcapsules. *Journal of Biomaterials Science, Polymer Edition*, *24*(9), 1084–1098.
32. Hueck, I. S., Haas, M., Finones, R., Frimodig, J., & Gough, D. A. (2011). The potential of selectively cultured adult stem cells re-implanted in tissues. In Artmann (Ed.), *Stem cell engineering: Principles and applications* (pp. 79–117). Berlin: Springer.
33. Seale, N. M., & Varghese, S. (2016). Biomaterials for pluripotent stem cell engineering: from fate determination to vascularization. *Journal of Materials Chemistry B, 4*(20), 3454–3463.
34. Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., & Yamanaka, S. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell, 131*(5), 861–872.
35. Aasen, T., Raya, A., Barrero, M. J., Garreta, E., Consiglio, A., Gonzalez, F., … Edel, M. (2008). Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nature Biotechnology*, *26*(11), 1276–1284.
36. Loh, Y. H., Hartung, O., Li, H., Guo, C., Sahalie, J. M., Manos, P. D., … Lensch, M. W. (2010). Reprogramming of T cells from human peripheral blood. *Cell Stem Cell, 7*(1), 15–19.
37. Zhou, T., Benda, C., Dunzinger, S., Huang, Y., Ho, J. C., Yang, J., … Bao, X. (2012). Generation of human induced pluripotent stem cells from urine samples. *Nature Protocols, 7* (12), 2080–2089.
38. Selvaraj, V., Plane, J. M., Williams, A. J., & Deng, W. (2010). Switching cell fate: the remarkable rise of induced pluripotent stem cells and lineage reprogramming technologies. *Trends in Biotechnology, 28*(4), 214–223.
39. Liu, H., Ye, Z., Kim, Y., Sharkis, S., & Jang, Y. Y. (2010). Generation of endoderm-derived human induced pluripotent stem cells from primary hepatocytes. *Hepatology, 51*(5), 1810–1819.
40. Sullivan, G. J., Hay, D. C., Park, I. H., Fletcher, J., Hannoun, Z., Payne, C. M., … Wang, G. (2010). Generation of functional human hepatic endoderm from human induced pluripotent stem cells. *Hepatology, 51*(1), 329–335.
41. Wu, S., Xu, H., Chen, B., Wen, Y., Ikusika, O. M., Ocker, A., … Ildstad, S. T. (2011). Sensitized recipients exhibit accelerated but not hyperacute rejection of vascularized composite tissue allografts. *Transplantation, 92*(6), 627–633.
42. Yamanaka, S. (2012). Induced pluripotent stem cells: Past, present, and future. *Cell Stem Cell, 10*(6), 678–684.
43. Pittenger, M. F., Mackay, A. M., Beck, S. C., Jaiswal, R. K., Douglas, R., Mosca, J. D., … Marshak, D. R. (1999). Multilineage potential of adult human mesenchymal stem cells. *Science, 284*(5411), 143–147.
44. Kadam, S., Muthyala, S., Nair, P., & Bhonde, R. (2010). Human placenta-derived mesenchymal stem cells and islet-like cell clusters generated from these cells as a novel source for stem cell therapy in diabetes. *The Review of Diabetic Studies, 7*(2), 168–182.
45. Ngoc, P. K., Van Phuc, P., Nhung, T. H., Thuy, D. T., & Nguyet, N. T. M. (2011). Improving the efficacy of type 1 diabetes therapy by transplantation of immunoisolated insulin-producing cells. *Human Cell, 24*(2), 86–95.
46. Goren, A., Dahan, N., Goren, E., Baruch, L., & Machluf, M. (2010). Encapsulated human mesenchymal stem cells: a unique hypoimmunogenic platform for long-term cellular therapy. *FASEB J, 24*(1), 22–31.
47. Wilson, J. L., & McDevitt, T. C. (2013). Stem cell microencapsulation for phenotypic control, bioprocessing, and transplantation. *Biotechnology and Bioengineering, 110*(3), 667–682.
48. Baraniak, P. R., & McDevitt, T. C. (2010). Stem cell paracrine actions and tissue regeneration. *Regenerative Medicine, 5*(1), 121–143.

49. Orive, G., Gascón, A. R., Hernández, R. M., Igartua, M., & Pedraz, J. L. (2003). Cell microencapsulation technology for biomedical purposes: Novel insights and challenges. *Trends in Pharmacological Sciences*, *24*(5), 207–210.
50. Hunkeler, D. (2001). Allo transplants xeno: As bioartificial organs move to the clinic. Introduction. *Annals of the New York Academy of Sciences, 944,* 1–6.
51. Kang, H. K., Wang, S., Dangi, A., Zhang, X., Singh, A., Zhang, L., … Thorp, E. B. (2017). Differential role of B cells and IL-17 versus IFN-gamma during early and late rejection of pig islet xenografts in mice. *Transplantation*.
52. Dufrane, D., & Gianello, P. (2012). Pig islet for xenotransplantation in human: Structural and physiological compatibility for human clinical application. *Transplantation Reviews, 26* (3), 183–188.
53. Dufrane, D., & Gianello, P. (2012). Macro- or microencapsulation of pig islets to cure type 1 diabetes. *World Journal of Gastroenterology, 18*(47), 6885–6893.
54. Zhu, H. T., Lu, L., Liu, X. Y., Yu, L., Lyu, Y., & Wang, B. (2015). Treatment of diabetes with encapsulated pig islets: An update on current developments. *Journal of Zhejiang University-Science B*, *16*(5), 329–343.
55. Gunzburg, W. H., & Salmons, B. (2000). Xenotransplantation: Is the risk of viral infection as great as we thought? *Molecular Medicine Today, 6*(5), 199–208.
56. Bowie, K. M., & Chang, P. L. (1998). Development of engineered cells for implantation in gene therapy. *Advanced Drug Delivery Reviews, 33*(1–2), 31–43.
57. Uludag, H., De Vos, P., & Tresco, P. A. (2000). Technology of mammalian cell encapsulation. *Advanced Drug Delivery Reviews, 42*(1–2), 29–64.
58. Sakai, S., Mu, C., Kawabata, K., Hashimoto, I., & Kawakami, K. (2006). Biocompatibility of subsieve-size capsules versus conventional-size microcapsules. *Journal of Biomedical Materials Research Part A, 78*(2), 394–398.
59. Fang, S., & MAO, L. (2007). Differentiation of embryoid-body cells derived from embryonic stem cells into hepatocytes in alginate microbeads in vitro. *Acta Pharmacologica Sinica*, *28*(12), 1924–1930.
60. Orive, G., Hernández, R. M., Gascón, A. R., Calafiore, R., Chang, T. M. S., de Vos, P., … Pedraz, J. L. (2004). History, challenges and perspectives of cell microencapsulation. *Trends in Biotechnology*, *22*(2), 87–92.
61. Rabanel, J. M., Banquy, X., Zouaoui, H., Mokhtar, M., & Hildgen, P. (2009). Progress technology in microencapsulation methods for cell therapy. *Biotechnology Progress*, *25*(4), 946–963.
62. Kidd, K. R., Nagle, R. B., & Williams, S. K. (2002). Angiogenesis and neovascularization associated with extracellular matrix-modified porous implants. *Journal of Biomedical Materials Research, 59*(2), 366–377.
63. Vasanthan, K. S., Subramanian, A., Krishnan, U. M., & Sethuraman, S. (2012). Role of biomaterials, therapeutic molecules and cells for hepatic tissue engineering. *Biotechnology Advances*, *30*(3), 742–752.
64. Maguire, T., Davidovich, A. E., Wallenstein, E. J., Novik, E., Sharma, N., Pedersen, H., … Yarmush, M. (2007). Control of hepatic differentiation via cellular aggregation in an alginate microenvironment. *Biotechnology and Bioengineering*, *98*(3), 631–644.
65. Shi, X. L., Zhang, Y., Gu, J. Y., & Ding, Y. T. (2009). Coencapsulation of hepatocytes with bone marrow mesenchymal stem cells improves hepatocyte-specific functions. *Transplantation, 88*(10), 1178–1185.
66. Gattazzo, F., Urciuolo, A., & Bonaldo, P. (2014). Extracellular matrix: A dynamic microenvironment for stem cell niche. *Biochimica et Biophysica Acta, 1840*(8), 2506–2519.
67. Vishwakarma, A., & Karp, J. M. (Eds.) (2017). *Biology and engineering of stem cell niches*. London: Elsevier Academic Press.
68. Jhala, D., & Vasita, R. (2015). A review on extracellular matrix mimicking strategies for an artificial stem cell niche. *Polymer Reviews, 55*(4), 561–595.
69. Scadden, D. T. (2006). The stem-cell niche as an entity of action. *Nature, 441*(7097), 1075–1079.

70. Stoker, M., & Gherardi, E. (1991). Regulation of cell movement: The motogenic cytokines. *Biochimica et Biophysica Acta, 1072*(1), 81–102.
71. Welf, E. S., & Haugh, J. M. (2011). Signaling pathways that control cell migration: Models and analysis. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine, 3*(2), 231–240.
72. Watt, F. M., & Huck, W. T. (2013). Role of the extracellular matrix in regulating stem cell fate. *Nature Reviews Molecular Cell Biology, 14*(8), 467–473.
73. Rowley, J. A., & Mooney, D. J. (2002). Alginate type and RGD density control myoblast phenotype. *Journal of Biomedical Materials Research, 60*(2), 217–223.
74. Benoit, D. S., Schwartz, M. P., Durney, A. R., & Anseth, K. S. (2008). Small functional groups for controlled differentiation of hydrogel-encapsulated human mesenchymal stem cells. *Nature Materials*, *7*(10), 816–823.
75. Chokkalingam, V., Tel, J., Wimmers, F., Liu, X., Semenov, S., Thiele, J., … Huck, W. T. (2013). Probing cellular heterogeneity in cytokine-secreting immune cells using droplet-based microfluidics. *Lab on a Chip*, *13*(24), 4740–4744.
76. Severian Dumitriu, V. P. (2013) *Medical and pharmaceutical applications, Vol. 2, 3rd ed.* ISBN 978-1-4200-9468-8.
77. Brandtner, D. (2013). *Bioencapsulation of living cells for diverse medical applications*. Bentham books. ISBN: 978-1-60805-721-4.
78. Strand, B. L., Ryan, L., Veld, P. I. T., Kulseng, B., Rokstad, A. M., Skjåk-Bræk, G., & Espevik, T. (2001). Poly-L-Lysine induces fibrosis on alginate microcapsules via the induction of cytokines. *Cell Transplant, 10*(3), 263–275.
79. Liu, Z. C., & Chang, T. M. (2002). Increased viability of transplanted hepatocytes when hepatocytes are co-encapsulated with bone marrow stem cells using a novel method. *Artificial Cells, Blood Substitutes, and Immobilization Biotechnology, 30*(2), 99–112.
80. Otterlei, M., Østgaard, K., Skjåk-Bræk, G., Smidsrød, O., Soon-Shiong, P., & Espevik, T. (1991). Induction of cytokine production from human monocytes stimulated with alginate. *Journal of Immunotherapy, 10*(4), 286–291.
81. Espevik, T., Otterlei, M., Skjåk-Braek, G., Ryan, L., Wright, S. D., & Sundan, A. (1993). The involvement of CD14 in stimulation of cytokine production by uronic acid polymers. *European Journal of Immunology, 23*(1), 255–261.
82. Aiedehe, K., Gianasii, E., Orienti, I., & Zecchi, V. (1997). Chitosan microcapsules as controlled release systems for insulin. *Journal of Microencapsulation*, *14*(5), 567–576.
83. Altiok, D., Altiok, E., & Tihminlioglu, F. (2010). Physical, antibacterial and antioxidant properties of chitosan films incorporated with thyme oil for potential wound healing applications. *Journal of Materials Science. Materials in Medicine, 21*(7), 2227–2236.
84. Green, D. W., Leveque, I., Walsh, D., Howard, D., Yang, X., Partridge, K., … Oreffo, R. O. (2005). Polysaccharide capsules for encapsulation, organization, and delivery of human cell types and growth factors. *Advanced Funtional Materials*, *15*(6), 917–923.
85. Krasaekoopt, W., Bhandari, B., & Deeth, H. (2004). The influence of coating materials on some properties of alginate beads and survivability of microencapsulated probiotic bacteria. *International Dairy Journal*, *14*(8), 737–743.
86. Chen, H., Ouyang, W., Jones, M., Metz, T., Martoni, C., Haque, T., … Prakash, S. (2007). Preparation and characterization of novel polymeric microcapsules for live cell encapsulation and therapy. *Cell Biochemistry and Biophysics*, *47*(1), 159–168.
87. Tan, W., Krishnaraj, R., & Desai, T. A. (2001). Evaluation of nanostructured composite collagen–chitosan matrices for tissue engineering. *Tissue Engineering, 7*(2), 203–210.
88. Chevallay, B., & Herbage, D. (2000). Collagen-based biomaterials as 3D scaffold for cell cultures: Applications for tissue engineering and gene therapy. *Medical and Biological Engineering and Computing, 38*(2), 211–218.

89. Malafaya, P. B., Silva, G. A., & Reis, R. L. (2007). Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. *Advanced Drug Delivery Reviews, 59*(4–5), 207–233.
90. Dautzenberg, H., Schuldt, U. T. E., Grasnick, G., Karle, P., MÜller, P., LÖhr, M., … Salmons, B. (1999). Development of cellulose sulfate-based polyelectrolyte complex microcapsules for medical applications. *Annals of the New York Academy of Sciences*, *875*, 46–63.
91. Swioklo, S., & Connon, C. J. (2016). Keeping cells in their place: The future of stem cell encapsulation. *Expert Opinion on Biological Therapy, 16*(10), 1181–1183.
92. Higgins, C. C. (1931). Solitary cysts of the kidney. *Annals of Surgery, 93*(4), 868–879.
93. Byass, P. (2014). The global burden of liver disease: A challenge for methods and for public health. *BMC Medicine, 12,* 159.
94. Hindley, C. J., Cordero-Espinoza, L., & Huch, M. (2016). Organoids from adult liver and pancreas: Stem cell biology and biomedical utility. *Development Biology, 420*(2), 251–261.
95. Fox, I. J., & Chowdhury, J. R. (2004). Hepatocyte transplantation. *American Journal of Transplantation, 4*(6), 7–13.
96. Sell, S. (2001). Heterogeneity and plasticity of hepatocyte lineage cells. *Hepatology, 33*(3), 738–750.
97. Miyajima, A., Tanaka, M., & Itoh, T. (2014). Stem/progenitor cells in liver development, homeostasis, regeneration, and reprogramming. *Cell Stem Cell, 14*(5), 561–574.
98. Itoh (2015). *Tissue-specific stem cell niche, stem cell biology and regeneration* ed. K. Turksen. Switzerland: Springer.
99. Conigliaro, A., Brenner, D. A., & Kisseleva, T. (2010). Hepatic progenitors for liver disease: Current position. *Stem Cells Cloning, 3,* 39–47.
100. Yang, W., Yan, H. X., Chen, L., Liu, Q., He, Y. Q., Yu, L. X., …. Chen, C. (2008). Wnt/beta-catenin signaling contributes to activation of normal and tumorigenic liver progenitor cells. *Cancer Research*, *68*(11), 4287–4295.
101. de Lau, W., Barker, N., Low, T. Y., Koo, B. K., Li, V. S., Teunissen, H., … Stange, D. E. (2011). Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. *Nature, 476*(7360). 293–297.
102. Boulter, L., Govaere, O., Bird, T. G., Radulescu, S., Ramachandran, P., Pellicoro, A., … Sansom, O. J. (2012). Macrophage-derived Wnt opposes notch signaling to specify hepatic progenitor cell fate in chronic liver disease. *Nature Medicine*, *18*(4), 572–579.
103. Huch, M., Dorrell, C., Boj, S. F., Van Es, J. H., Li, V. S., Van De Wetering, M., … Haft, A. (2013). In vitro expansion of single Lgr5 + liver stem cells induced by Wnt-driven regeneration. *Nature, 494*(7436), 247–250.
104. Xinguang, Y., Huixing, Y., Xiaowei, W., Xiaojun, W., & Linghua, Y. (2015). R-spondin1 arguments hepatic fibrogenesis in vivo and in vitro. *Journal of Surgical Research*, *193*(2), 598–605.
105. Kopp, J. L., Grompe, M., & Sander, M. (2016). Stem cells versus plasticity in liver and pancreas regeneration. *Nature Cell Biology, 18*(3), 238–245.
106. Yanger, K., Knigin, D., Zong, Y., Maggs, L., Gu, G., Akiyama, H., … Stanger, B. Z. (2014). Adult hepatocytes are generated by self-duplication rather than stem cell differentiation. *Cell Stem Cell, 15*(3), 340–349.
107. Tarlow, B. D., Pelz, C., Naugler, W. E., Wakefield, L., Wilson, E. M., Finegold, M. J., & Grompe, M. (2014). Bipotential adult liver progenitors are derived from chronically injured mature hepatocytes. *Cell Stem Cell, 15*(5), 605–618.
108. Ji, J., & Wang, X. W. (2012). Clinical implications of cancer stem cell biology in hepatocellular carcinoma. *Seminars in Oncology, 39*(4), 461–472.
109. Di Bonzo, L. V., Ferrero, I., Cravanzola, C., Mareschi, K., Rustichell, D., Novo, E., … Davit, A. (2008). Human mesenchymal stem cells as a two-edged sword in hepatic

regenerative medicine: Engraftment and hepatocyte differentiation versus profibrogenic potential. *Gut, 57*(2), 223–231.
110. Cai, J., Zhao, Y., Liu, Y., Ye, F., Song, Z., Qin, H., … Guo, Y. (2007). Directed differentiation of human embryonic stem cells into functional hepatic cells. *Hepatology, 45* (5), 1229–1239.
111. Basma, H., Soto–Gutiérrez, A., Yannam, G. R., Liu, L., Ito, R., Yamamoto, T., … Muirhead, D. (2009). Differentiation and transplantation of human embryonic stem cell-derived hepatocytes. *Gastroenterology, 136*(3), 990–999.
112. Touboul, T., Hannan, N. R., Corbineau, S., Martinez, A., Martinet, C., Branchereau, S., … Weber, A. (2010). Generation of functional hepatocytes from human embryonic stem cells under chemically defined conditions that recapitulate liver development. *Hepatology, 51*(5), 1754–1765.
113. Magyar, J. P., Nemir, M., Ehler, E., Suter, N., Perriard, J. C., & Eppenberger, H. M. (2001). Mass production of embryoid bodies in microbeads. *Annals of the New York Academy of Sciences, 944*(1), 135–143.
114. Cho, N. J., Elazar, M., Xiong, A., Lee, W., Chiao, E., Baker, J., … Glenn, J. S. (2008). Viral infection of human progenitor and liver-derived cells encapsulated in three-dimensional PEG-based hydrogel. *Biomedical materials*, *4*(1), 011001.
115. Meier, R. P., Mahou, R., Morel, P., Meyer, J., Montanari, E., Muller, Y. D., … Bühler, L. H. (2015). Microencapsulated human mesenchymal stem cells decrease liver fibrosis in mice. *Journal of Hepatology*, *62*(3), 634–641.
116. Meier, R. P., Montanari, E., Morel, P., Pimenta, J., Schuurman, H. J., Wandrey, C., … Bühler, L. H. (2017). Microencapsulation of hepatocytes and mesenchymal stem cells for therapeutic applications. *Hepatocyte Transplantation: Methods and Protocols,* 259–271.
117. Kisseleva, T., Uchinami, H., Feirt, N., Quintana-Bustamante, O., Segovia, J. C., Schwabe, R. F., & Brenner, D. A. (2006). Bone marrow-derived fibrocytes participate in pathogenesis of liver fibrosis. *Journal of hepatology, 45*(3), 429–438.
118. Maguire, T., Novik, E., Schloss, R., & Yarmush, M. (2006). Alginate-PLL microencapsulation: Effect on the differentiation of embryonic stem cells into hepatocytes. *Biotechnology and Bioengineering*, *93*(3). 581–591.
119. Fang, S., & MAO, L. (2007). Differentiation of embryoid-body cells derived from embryonic stem cells into hepatocytes in alginate microbeads in vitro. *Acta Pharmacologica Sinica, 28*(12), 1924–1930.
120. Palakkan, A. A., Hay, D. C., TV, K., & Ross, J. A. (2013). Liver tissue engineering and cell sources: Issues and challenges. *Liver International*, *33*(5), 666–676.
121. Kirk, K., Hao, E., Lahmy, R., & Itkin-Ansari, P. (2014). Human embryonic stem cell derived islet progenitors mature inside an encapsulation device without evidence of increased biomass or cell escape. *Stem Cell Research*, *12*(3), 807–814.
122. Scharp, D. W., & Marchetti, P. (2014). Encapsulated islets for diabetes therapy: History, current progress, and critical issues requiring solution. *Advanced Drug Delivery Reviews, 67–68,* 35–73.
123. Sakata, N., Sumi, S., Yoshimatsu, G., Goto, M., Egawa, S., & Unno, M. (2012). Encapsulated islets transplantation: Past, present and future. *World Journal of Gastrointestinal Pathophysiology, 3*(1), 19–26.
124. Kirchhof, N., Shibata, S., Wijkstrom, M., Kulick, D. M., Salerno, C. T., Clemmings, S. M., … Hering, B. J. (2004). Reversal of diabetes in non-immunosuppressed rhesus macaques by intraportal porcine islet xenografts precedes acute cellular rejection. *Xenotransplantation, 11* (5), 396–407.
125. Elliott, R. B., Escobar, L., Tan, P. L. J., Garkavenko, O., Calafiore, R., Basta, P., … Bambra, C. (2005, October). Intraperitoneal alginate-encapsulated neonatal porcine islets in a

placebo-controlled study with 16 diabetic cynomolgus primates. *Transplantation Proceedings, 37*(8), 3505–3508.
126. Duvivier-Kali, V. F., Omer, A., Lopez-Avalos, M. D., O'neil, J. J., & Weir, G. C. (2004). Survival of microencapsulated adult pig islets in mice in spite of an antibody response. *American Journal of Transplantation*, *4*(12), 1991–2000.
127. Elliott, R. B., Escobar, L., Tan, P. L., Muzina, M., Zwain, S., & Buchanan, C. (2007). Live encapsulated porcine islets from a type 1 diabetic patient 9.5 yr after xenotransplantation. *Xenotransplantation, 14*(2), 157–161.
128. Tuch, B. E., Keogh, G. W., Williams, L. J., Wu, W., Foster, J. L., Vaithilingam, V., & Philips, R. (2009). Safety and viability of microencapsulated human islets transplanted into diabetic humans. *Diabetes Care, 32*(10), 1887–1889.
129. King, A., Lau, J., Nordin, A., Sandler, S., & Andersson, A. (2003). The effect of capsule composition in the reversal of hyperglycemia in diabetic mice transplanted with microencapsulated allogeneic islets. *Diabetes Technology & Therapeutics, 5*(4), 653–663.
130. King, A., Andersson, A., Strand, B. L., Lau, J., Skjåk-Bræk, G., & Sandler, S. (2003). The role of capsule composition and biologic responses in the function of transplanted microencapsulated islets of Langerhans. *Transplantation, 76*(2), 275–279.
131. Kulseng, B., Thu, B., Espevik, T., & Skjåk-Bræk, G. (1997). Alginate polylysine microcapsules as immune barrier: Permeability of cytokines and immunoglobulins over the capsule membrane. *Cell Transplant, 6*(4), 387–394.
132. Juste, S., Lessard, M., Henley, N., Ménard, M., & Hallé, J. P. (2005). Effect of poly-L-lysine coating on macrophage activation by alginate-based microcapsules: Assessment using a new in vitro method. *Journal of Biomedical Materials Research Part A*, *72*(4), 389–398.
133. Pueyo, M. E., Darquy, S., Capron, F., & Reach, G. (1994). In vitro activation of human macrophages by alginate-polylysine microcapsules. *Journal of Biomaterials Science, Polymer Edition*, *5*(3), 197–203.
134. Brennan, D. C., Shannon, M. B., Koch, M. J., Polonsky, K. S., Desai, N., & Shapiro, J. (2004). Portal vein thrombosis complicating islet transplantation in a recipient with the Factor V Leiden mutation. *Transplantation, 78*(1), 172–173.
135. Kawahara, T., Kin, T., Kashkoush, S., Gala-Lopez, B., Bigam, D. L., Kneteman, N. M., … Shapiro, A. J. (2011). Portal vein thrombosis is a potentially preventable complication in clinical islet transplantation. *American Journal of Transplantation*, *11*(12), 2700-2707.
136. Sakata, N., Tan, A., Chan, N., Obenaus, A., Mace, J., Peverini, R., … Hathout, E. (2009, February). Efficacy comparison between intraportal and subcapsular islet transplants in a murine diabetic model. *Transplantation Proceedings*, *41*(1), 346–349.
137. Christoffersson, G., Carlsson, P. O., & Phillipson, M. (2011). Intramuscular islet transplantation promotes restored islet vascularity. *Islets, 3*(2), 69–71.
138. McQuilling, J. P., Arenas-Herrera, J., Childers, C., Pareta, R. A., Khanna, O., Jiang, B., … Opara, E. C. (2011, November). New alginate microcapsule system for angiogenic protein delivery and immunoisolation of islets for transplantation in the rat omentum pouch. *Transplantation Proceedings*, *43*(9), 3262–3264.
139. Elliott, R. B., Escobar, L., Calafiore, R., Basta, G., Garkavenko, O., Vasconcellos, A., et al. (2005). Transplantation of micro- and macroencapsulated piglet islets into mice and monkeys. *Transplantation Proceedings, 37*(1), 466–469.
140. Dufrane, D., van Steenberghe, M., Goebbels, R. M., Saliez, A., Guiot, Y., & Gianello, P. (2006). The influence of implantation site on the biocompatibility and survival of alginate encapsulated pig islets in rats. *Biomaterials, 27*(17), 3201–3208.
141. Algire, G. (1943). An adaptation of the transparent-chamber technique to the mouse. *Journal of the National Cancer Institute, 1943*(4), 1–11.
142. Storrs, R., Dorian, R., King, S. R., Lakey, J., & Rilo, H. (2001). Preclinical development of the Islet sheet. *Annals of the New York Academy of Sciences, 2001*(944), 252–266.

143. Nyitray, C. E., Chavez, M. G., & Desai, T. A. (2014). Compliant 3D microenvironment improves beta-cell cluster insulin expression through mechanosensing and beta-catenin signaling. *Tissue Engineering Part A, 20*(13–14), 1888–1895.
144. de Vos, P., Hamel, A. F., & Tatarkiewicz, K. (2002). Considerations for successful transplantation of encapsulated pancreatic islets. *Diabetologia, 45*(2), 159–173.
145. Veriter, S., Aouassar, N., Xhema, D., Beaurin, G., Adnet, P. Y., Igarashi, Y., … Dufrane, D. (2011). Islets and mesenchymal stem cells co-encapsulation can improve subcutaneous bioartificial pancreas survival in diabetic primates. The Transplantation Society: CTS-IXA. Parallel Session 5—ISLET xenotransplantation—Preclinical models.
146. Davis, N. E., Beenken-Rothkopf, L. N., Mirsoian, A., Kojic, N., Kaplan, D. L., Barron, A. E., & Fontaine, M. J. (2012). Enhanced function of pancreatic islets co-encapsulated with ECM proteins and mesenchymal stromal cells in a silk hydrogel. *Biomaterials, 33*(28), 6691–6697.
147. Lee, S. H., Hao, E., Savinov, A. Y., Geron, I., Strongin, A. Y., & Itkin-Ansari, P. (2009). Human beta-cell precursors mature into functional insulin-producing cells in an immunoisolation device: Implications for diabetes cell therapies. *Transplantation, 87*(7), 983–991.
148. Matveyenko, A. V., Georgia, S., Bhushan, A., & Butler, P. C. (2010). Inconsistent formation and nonfunction of insulin-positive cells from pancreatic endoderm derived from human embryonic stem cells in athymic nude rats. *American Journal of Physiology-Endocrinology and Metabolism, 299*(5), E713–E720.
149. Colton, C. K. (2014). Oxygen supply to encapsulated therapeutic cells. *Advanced Drug Delivery Reviews, 67–68,* 93–110.
150. Candiello, J., Singh, S. S., Task, K., Kumta, P. N., & Banerjee, I. (2013). Early differentiation patterning of mouse embryonic stem cells in response to variations in alginate substrate stiffness. *Journal of Biological Engineering*, *7*(1), 9.
151. Schulz, T. C. (2015). Concise review: Manufacturing of pancreatic endoderm cells for clinical trials in Type 1 diabetes. *Stem Cells Translational Medicine, 4*(8), 927–931.
152. Pagliuca, F. W., Millman, J. R., Gürtler, M., Segel, M., Van Dervort, A., Ryu, J. H., … Melton, D. A. (2014). Generation of functional human pancreatic beta cells in vitro. *Cell, 159*(2), 428–439.
153. Rezania, A., Bruin, J. E., Arora, P., Rubin, A., Batushansky, I., Asadi, A., … Yang, Y. H. C. (2014). Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. *Nature Biotechnology*, *32*(11), 1121–1133.
154. Russ, H. A., Parent, A. V., Ringler, J. J., Hennings, T. G., Nair, G. G., Shveygert, M., … Blelloch, R. (2015). Controlled induction of human pancreatic progenitors produces functional beta-like cells in vitro. *The EMBO Journal*, *34*(13), 1759–1772.
155. Agulnick, A. D., Ambruzs, D. M., Moorman, M. A., Bhoumik, A., Cesario, R. M., Payne, J. K., …Kerr, J. (2015). Insulin-producing endocrine cells differentiated in vitro from human embryonic stem cells function in macroencapsulation devices in vivo. *Stem Cells Translational Medicine*, *4*(10), 1214–1222.
156. Tateishi, K., He, J., Taranova, O., Liang, G., D'Alessio, A. C., & Zhang, Y. (2008). Generation of insulin-secreting islet-like clusters from human skin fibroblasts. *Journal of Biological Chemistry*, *283*(46), 31601–31607.
157. Maehr, R., Chen, S., Snitow, M., Ludwig, T., Yagasaki, L., Goland, R., … Melton, D. A. (2009). Generation of pluripotent stem cells from patients with type 1 diabetes. *Proceedings of the National Academy of Sciences*, *106*(37), 15768–15773.
158. Lysy, P. A., Weir, G. C., & Bonner-Weir, S. (2012). Concise review: pancreas regeneration: Recent advances and perspectives. *Stem Cells Translational Medicine, 1*(2), 150–159.
159. Noguchi, H., Xu, G., Matsumoto, S., Kaneto, H., Kobayashi, N., Bonner-Weir, S., & Hayashi, S. (2006). Induction of pancreatic stem/progenitor cells into insulin-producing cells by adenoviral-mediated gene transfer technology. *Cell transplantation*, *15*(10), 929–938.
160. Alberto Hayek, C.K., Brief review: Cell replacement therapies to treat type 1 diabetes mellitus. *Clinical Diabetes and Endocrinology, 2*(4).

161. Mason, M. N., & Mahoney, M. J. (2009). Selective beta-cell differentiation of dissociated embryonic pancreatic precursor cells cultured in synthetic polyethylene glycol hydrogels. *Tissue Engineering Part A, 15*(6), 1343–1352.
162. D'Amour, K. A., Bang, A. G., Eliazer, S., Kelly, O. G., Agulnick, A. D., Smart, N. G., … Baetge, E. E. (2006). Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nature Biotechnology*, *24*(11), 1392–1401.
163. Kroon, E., Martinson, L. A., Kadoya, K., Bang, A. G., Kelly, O. G., Eliazer, S., … Agulnick, A. D. (2008). Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. *Nature Biotechnology*, *26*(4), 443–452.
164. Chen, Y., Pan, F. C., Brandes, N., Afelik, S., Sölter, M., & Pieler, T. (2004). Retinoic acid signaling is essential for pancreas development and promotes endocrine at the expense of exocrine cell differentiation in Xenopus. *Developmental Biology*, *271*(1), 144–160.
165. Gomes, L. F., Lorente, S., Simon-Giavarotti, K. A., Areco, K. N., Araújo-Peres, C., & Videla, L. A. (2004). Tri-iodothyronine differentially induces Kupffer cell ED1/ED2 subpopulations. *Molecular Aspects of Medicine*, *25*(1–2), 183–190.
166. Schulz, T. C., Young, H. Y., Agulnick, A. D., Babin, M. J., Baetge, E. E., Bang, A. G., … Kadoya, K. (2012). A scalable system for production of functional pancreatic progenitors from human embryonic stem cells. *PLoS One*, *7*(5), e37004.
167. Shapiro, A. M., Nanji, S. A., & Lakey, J. R. (2003). Clinical islet transplant: Current and future directions towards tolerance. *Immunological Reviews, 196,* 219–236.
168. Wang, T., Lacík, I., Brissová, M., Anilkumar, A. V., Prokop, A., Hunkeler, D., … Powers, A. C. (1997). An encapsulation system for the immunoisolation of pancreatic islets. *Nature Biotechnology*, *15*(4), 358–362.
169. Desai, T., & Shea, L. D. (2017). Advances in islet encapsulation technologies. *Nature Reviews Drug Discovery, 16*(5), 367.
170. Brauker, J. H., Carr-Brendel, V. E., Martinson, L. A., Crudele, J., Johnston, W. D., & Johnson, R. C. (1995). Neovascularization of synthetic membranes directed by membrane microarchitecture. *Journal of Biomedical Materials Research Part A, 29*(12), 1517–1524.
171. Geller, R. L., Loudovaris, T., Neuenfeldt, S., Johnson, R. C., & Brauker, J. H. (1997). Use of an immunoisolation device for cell transplantation and tumor immunotherapy. *Annals of the New York Academy of Sciences, 1997*(831), 438–451.
172. Colton, C. K., & Avgoustiniatos, E. S. (1991). Bioengineering in development of the hybrid artificial pancreas. *Journal of Biomechanical Engineering, 113*(2), 152–170.
173. Su, J., Hu, B. H., Lowe, W. L., Kaufman, D. B., & Messersmith, P. B. (2010). Anti-inflammatory peptide-functionalized hydrogels for insulin-secreting cell encapsulation. *Biomaterials, 31*(2), 308–314.
174. Rafael, E., Wu, G. S., Hultenby, K., Tibell, A., & Wernerson, A. (2003). Improved survival of macroencapsulated islets of Langerhans by preimplantation of the immunoisolating device: A morphometric study. *Cell Transplantation*, *12*(4), 407–412.
175. Yakhnenko, I., Wong, W. K., Katkov, I. I., & Itkin-Ansari, P. (2012). Cryopreservation of human insulin expressing cells macro-encapsulated in a durable therapeutic immunoisolating device theracyte. *Cryo Letters, 33*(6), 518–531.
176. Rafael, E., Wernerson, A., Arner, P., Wu, G. S., & Tibell, A. (1999). In vivo evaluation of glucose permeability of an immunoisolation device intended for islet transplantation: A novel application of the microdialysis technique. *Cell Transplantation*, *8*(3), 317–326.
177. Kumagai-Braesch, M., Jacobson, S., Mori, H., Jia, X., Takahashi, T., Wernerson, A., … Tibell, A. (2013). The TheraCyte device protects against islet allograft rejection in immunized hosts. *Cell Transplantation*, *22*(7), 1137–1146.
178. ClinicalTrials.gov/beta/. (2016). ViaCyte: Three Year Follow-up Safety Study in Subjects Previously Implanted With VC-01™.

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