Biofabrication of the human pancreas for the treatment of diabetes mellitus

Abstract

Diabetes mellitus (Type 1 Diabetes or T1D) is an autoimmune disease that results in the destruction of the insulin-producing beta cells of the pancreas. Decreased levels of insulin in the blood topple homeostatic balances established by negative feedback, preventing the conversion of monosaccharides in the blood to polysaccharides in cells. Current treatment of *T1D* requires calculated, sporadic injections of insulin: a temporary treatment at best. A more permanent treatment for *T1D* requires a pancreas transplant, subject to organ rejection and medical unavailability. New technologies allow for the construction of certain simple tissues in a method known as bioprinting. We propose to use the newly developing processes of organ bioprinting from a patient's own monopotent stem cells to "print" an artificial pancreas which will utilise cell encapsulation to affect the permeability of the pancreas to cytotoxic T-cells, and produce biologically compatible beta cells unaffected by autoimmune targeting.

Biofabrication of the human pancreas for the treatment of diabetes mellitus

Present Technology

The process of "bioprinting" is a relatively recent field of science and technology. It involves the utilization of modified 3D printers to fabricate living human tissue. Present printing technology is used to deposit living cells, along with a host of other organic compounds (mainly proteins and drugs), onto a solid or gel substrate. There are currently several methods known in which technology exists to be able to deposit cells on a substrate. We will be focusing primarily on "acoustic printing".

Acoustic printing is preferable to other methods of printing as: a) no nozzle is required to form liquid droplets while they are produced from an open reservoir, ameliorating complications that may arise from clogging or cell damage; b) unlike laser-guided printing, a process that harms cells due to excessive thermal energy, acoustic waves do not harm cells. The pulse durations last only for a few microseconds, insignificant time to cause any cell damage; c) multiple acoustic ejectors can be combined into an array, allowing the fabrication of several different biomaterials at once; and d) acoustic printing has the highest throughput of all known fabrication methods and can eject droplets as small as 3μm in diameter.

The most common technique utilized in the creation of lab-grown organs involves fabricating a polyester scaffold and layering cells onto said scaffold. This can take months to complete. By comparison, biofabrication offers greater speeds and a higher degree of precision in the placement of cells. Lab grown hollow organs, such as the stomach and bladder, have already been successfully transplanted into patients.

However, there is an oversight. These scaffolds degrade, eliciting immunogenic reactions

as they deform. One of the key challenges of designing a viable tissue and organ biofabrication method is to encourage the natural self-organization of cells. It is virtually impossible to precisely print each individual microvessel, some of which are only one cell thick. For this reason, the use of a stem-cell print sample is recommended. Stem cells demonstrate multilineage differentiation which may propagate cells rarely found in the body. Since stem cells are taken from the patient themselves, the chances of tissue rejection would be minimal. Because of such, stem cells allow for a greater versatility in the creation and transplantation of biofabricated organs.

History

In 1984, Charles Hull invented stereolithography: a method of fabricating solid, plastic 3D objects. In this process, an ultraviolet laser is shone on a light-sensitive liquid polymer causing it to solidify, layer by layer. These layers stick to each other, forming the completed, fabricated product. The development of this process was essential for the advancement of the relatively new field of bioprinting.

Bioprinting originated in 2000, when Dr. Thomas Boland and his colleagues modified an inkjet printer to print a variation of *E. coli* bacteria [7]. Inkjet printers launch tiny droplets of ink on paper resulting in a printed page. The variations designed by Boland were modified to launch microscopic prokaryotic cells. Due to his success, he then altered this printer once more in order to print larger, eukaryotic animal cells from rats. In 2003, after creating a printer that had approximately a 90% survival rate for printed cells, he filed the first patent for the “process of bioprinting” [7]. There were limitations with his printer: it could only print two-dimensional

biological structures without of the complexity of true organs.

Future innovations in the field of 3D printing involved integration into the field of medicine. The prototypes first utilized in medical bioprinting functioned by outputting layers of cells on top of each other, using artificial scaffolds as substrate. The cells are protected with a dissolvable gel during printing, but it is still a challenge to ensure that most of the cells survive the printing process. This combination of cells and gel is called the “bio-ink” of the printer [2].

These artificial scaffolds have certain requirements. Diffusion needs to occur between cells through the scaffold, which would require a certain porosity and biodegradability. Biodegradability is important: the surrounding tissue would absorb the scaffold instead of requiring an infeasible surgical removal. These dissolved scaffold materials would also have to be benign to other existing systems in the body.

In recent years, the field of bioprinting has observed many advances. In 2010, the first bioprinted blood vessel was constructed by Organovo, a pioneering bioprinting startup [9]. Their printer, called the NovoGen MMX, was used to print the blood vessel and corresponding cardiac tissue. This printer had three printheads that each served different purposes: One head responsible for printing cardiac cells, another for endothelial cells, and another for the collagen scaffold that supported the cells. The creation of the NovoGen MMX was significant in that it bioprinted the scaffold, which hastened production.

The NovoGen MMX also demonstrated that cells do not need to be placed in precise locations, since they tend to migrate to the correct regions [9]. For example, in the printed blood vessel, endothelial cells are moved inside the vessel. This is not surprising since cells in an

embryo arrange themselves naturally to form organs. Bioprinters cannot accurately print out capillaries and thus natural cell self-organization is required in order to provide the maximum diffusion distance of 0.01mm to ensure cell viability.

Future Technology

Bioprinting is a potential industry with great incentive for monetary investment. With recent breakthroughs including the successful bioprinting of a liver, the bioprinting of the pancreas in the next 20 years seems very feasible. In impending years, bioprinting could have the same scientific popularity and appeal as *penicillin* in the mid 1900s.

Organ transplantation is a medical practice that has a severe deficiency in resources. Currently, when a patient requires an organ transplant, he/she often has to have the transplant delayed for an indefinite period of time. The only solution to this delay is to directly receive the organ from a family member, which can only be done with nonessential parts of organs, such as the kidney or a portion of the liver [3].

In the future, the alternative to natural transplantation is bioprinting. Bioprinting, in this sense, will not only model individual cells or tissues, but also organs. The reason today in which organs cannot be biofabricated is that while certain cells or tissue of an organ are being bioprinted, the other parts fail to become vascularized and die due to gangrene.

The bioprinter should also introduce tremendous technological advancements that would consequently make feasible the bioprinting of an organ through the natural connection of tissues. The current leading technology: Organovo and Invetech's Novogen MMX bioprinter, has

performed remarkable feats, such as the the bioprinting of a mini-liver and various human tissues. However, this was designed to print tissue, not full organs. Until the speed or range of the bioprinter improves, vascularization between various parts of an organ remains difficult to achieve. However, with time, this technology should naturally improve to facilitate these wider specifications [9].

Bioprinting of organs can also exponentially accelerate the clinical trial process. Instead of having to test a new drug on a mouse or human, which time-consuming and dangerous (to both mice and humans) [1], we can observe its effect on a specific tissue of one's organ. Therefore, the range of clinical trials that can be run with bioprinted tissue is limited, as only one organ is targeted, but with even more advanced bioprinting technology, this can be expanded.

In twenty years, we predict that treatment of *Diabetes mellitus* (*type 1 diabetes or T1D),* with the transplantation of bioprinted pancreases, will be in the clinical trial process. If any further complications, such as the rejection of the pancreases or a further autoimmune response to insulin-producing beta cells, arise with this treatment, the possibility of utilizing transplanted pancreases in treatment will fail. Hopefully, this will not be the case, but there is always a significant risk to treating any disease of this severity.

The pancreas is one of the most critical organs to undergo a consideration for bioprinting. *Type 1* diabetics undergo tedious treatment simply to survive with their disease. When diabetes becomes extremely severe, leading to renal failure, both the pancreas and the kidney must be replaced. These patients often have trouble acquiring the organs; the average wait time for a pancreas transplant is about two years [10]. In addition, a kidney could be donated by a living

family member, but a pancreas can only be transplanted from a cadaver. Therefore, in the future, both the kidney and the pancreas can be bioprinted, but given the kidney's complex physiology, the pancreas will likely be bioprinted sooner.

In the future, the goal is to biofabricate enough pancreases so they are accessible to the entire *T1D* population. However, transplants using a bioprinted pancreas can only be performed if the kidney is functional (because of the overworking of the renal gland during the surgery) in the type-1 patient. If not, a kidney transplant is also necessary. Therefore, transplants for some type-1 patients (in very severe cases) may only be feasible once both organs can be bioprinted. Until this technology comes about, the bioprinting of islet cells in the pancreas (that produce beta cells that produce insulin) can be done. However, these cells are also taken from a cadaver's pancreas, so these islet cells are still inaccessible to a vast majority of the *T1D* population [14].

Breakthroughs

Traditionally, the practice of bio-structural engineering utilizes "artificial adhesive scaffolds" to seed and place cells. Scaffolding involves the use of degradable porous scaffolds or hydrogels as a print base to produce thicker tissue. Though this has allowed for more organ-like printing processes, the process is problematic as the degradation of these scaffolds can elicit dangerous immunogenic reactions. Since we aim to design and print a fully functional organ using a process that does not utilize scaffolding, advancements in bio-manufacturing technology must be realized prior to further development.

One of the biggest setbacks is the the lack of vascularization in biofabricated organs. Capillaries can exist in beds surrounding all cells in the body at a distance of no more than 2 mm to ensure adequate cellular exchange of nutrients and metabolites. At the smallest, capillaries are

a single cell wide (8 μm) surrounded by vascular tissue. Currently, 3D printers are not advanced enough to print at such a fine detail or the complexity necessary (different tissue types), which ultimately results in mass programmed cell death (apoptosis). In past attempts to bioprint organs, existing cells became malnourished while similar cells were being printed simultaneously.

The process of bio-structural engineering is capable of printing such tissue at the moment, but is not capable of printing them functionally. It has to include the vascular network of blood vessels to supply nutrients and minerals to the cells. Generally the maximum possible distance for oxygen diffusion is 0.2mm, but necrosis may occur when the diffusion distance exceeds 0.01μm [8]. The challenge becomes much more difficult in large, complex organs such as the kidney.

In November 2013, Organovo, a San Diego-based startup, created a functional mini-liver that survived 40 days [6]. This was done by scaffolding hepatocytes and endothelial cells, which replicated until they formed a human liver. Hepatocytes have very short lifespans outside of the human body, so the lifespan of this liver was very surprising. However, this liver was only a fraction of the size of the adult human liver, and like most bioprinted organs, lacked the ability to vascularize, and therefore was not functional in the short term.

One way in which bioprinted organs could be vascularized is through the bioprinting of blood vessels. To bioprint the organ, the various tissues need to be preserved separately while the blood vessels connect them. However, this process is currently problematic: blood vessels, especially cell-wide capillaries, are currently impossible to print because blood vessels in their anatomy are a vast network of tissues.

The issue of autoimmune damage to islet cells could also be alleviated by the use of islet

cell encapsulation. The cells are encapsulated with the ECM proteins laminin and collagen IV, which acts as a barrier preventing damage by white blood cells. Smaller particles such as insulin and oxygen could bypass this membrane but larger particles such as the white blood cell cannot. In addition, the encapsulated cells have increased gene expression in insulin I, insulin II, glucagon, and somatostatin compared to non-encapsulated cells. A 3.2-fold improvement in islet insulin secretion was observed when islets were co-encapsulated with MSCs and ECM proteins [4].This means the efficiency and lifespan of islet cells could be greatly enhanced by encapsulating them.

Design Process

We ruled out three ideas before choosing the bioprinting of a pancreas as our project. Before we decided on our idea, we were interested in the concept of bioprinting organs. We see it as a fascinating concept with the potential to tremendously improve our current system of organ transplants, but the first idea we ruled out was bioprinting a kidney. Since a kidney transplant is of great importance, we believed that bioprinting the kidney would be of great use to society. Although this may be accomplished in sometime in the future, the kidney is an extremely complex organ in anatomy and physiology that would take much longer to develop. Thus, it was beyond the scope of our project. We wanted to create a proof of concept for the feasibility of bioprinting organs and so the pancreas, being a simpler organ, is a much more viable option to research.

The next idea we also ruled out was the bioprinting of a liver. When we discovered that bioprinting the kidney in 20 years was unreasonable, we turned to another organ: the liver. We believed the liver was better capable of being 3D bioprinted because of its natural regeneration

properties. Only a portion of the liver would have to be bioprinted and vascularized, making it the most efficient organ to be bioprinted. But while we considered the liver, we had also taken other organs into account, primarily the pancreas. While liver transplants are common and in great need, a pancreas can only be transplanted from a cadaver, thus limiting the number of available pancreases for transplant. Therefore, we saw a greater need to bioprint an organ such as the pancreas in which there is a fatally long wait list for transplantation. In addition, we made our final decision immediately after Organovo released news of its bioprinted liver that survived for 40 days. With the combination of the fact that the bioprinting of the liver may take far less than 20 years, and that pancreas transplants are in greater necessity, we opted for the latter.

The last topic we ruled out was the stem cell differentiation of islet cells to treat *T1D* as an alternative to the pancreas transplant . Islet cell transplantation is the transplantation of islet cells from the pancreas from one person into another. Barring an autoimmune response, those cells generate the beta cells that produce the hormones needed to naturally regulate cell storage of glucose. Initially, this topic seemed more appealing than pancreas transplantation since the process seemed much less arduous. The problem with this method of treating *T1D* is the limited supply of islet cells available for transplantation.

One solution to this would be to differentiate the patients own stem cells into islet cells, but we discovered that there was another immense problem with this method: an autoimmune response often follows the transplantations. Since *T1D* is an autoimmune disorder, new islet cells will still be attacked by the body's autoimmune system. A likely candidate for the reaction is the P2RX7 pathway. Studies have been done that suggest the pathway is an early trigger for the autoimmune response that destroys islet cells [12]. However, the direct cause of *T1D* is still

unclear and patients, after these transplantations, still have to take immunosuppressant medications for an indefinite period of time. These medications may have severe side effects, most commonly of which is renal failure. When immunosuppressants are taken for long periods of time, which is the case for many patients that undergo islet cell transplantation, we believe the detriments outweigh the benefits.

Consequences

The ability to bioprint a pancreas would improve the lives of countless people suffering from *T1D*. Successful bioprinting of the pancreas would provide a readily available alternative to harvesting organs from cadavers. However, there are many considerations and consequences behind such a development.

Drawbacks of bioprinting include the potential over secretion of hormones such as insulin. Currently, there are more patients requiring pancreas transplantation than there are available pancreases, and the average wait time for a transplant is over two years [10]. A bioprinted pancreas can greatly reduce wait times, especially once the bioprinting process allows for the rapid substitution of organic transplantation. Furthermore, the pancreas rejection rate after a transplant is 55% after five years [3], since there is sometimes an autoimmune response to the foreign tissue. This means that for many patients, a pancreas transplant is only a temporary solution. The rejection rate could be reduced to near 0% as: a) the bioprinted pancreas uses the patient's own pancreatic unipotent stem cells to ensure the antibodies do not attack the pancreas; and b) the islet cells would be encapsulated by various proteins to protect them from the autoimmune system [13]. The development of a bioprinted pancreas would also benefit people with *T1D*. *T1D* is an autoimmune disorder in which the immune system attacks insulin

producing beta cells in the pancreas. A pancreas transplant is often necessary for patients with severe cases of *T1D*, but could also be beneficial and cost-effective to anyone with the disease. Currently, insulin is costly, and thus unaffordable to many regions of the world [5]. Eventually, this accumulates to tens of thousands of dollars a month, making long-term insulin treatment more expensive than a singular pancreas transplant, with the cost of a bioprinted pancreas decreasing over time as efficiency of bioprinters is improved. This would mark the advent of more advanced techniques necessary for the development and bio-structural engineering of complex organs.

Another potential drawback of a bioprinted pancreas is the over-secretion of hormones. Recently, researchers have found a correlation between high insulin levels and an increase in heart failure incidences [11]. Faulty negative feedback may cause an excess of insulin, which may cause blood sugar levels to drop. Other hormones levels must also be regulated in order to avoid negative effects on the body, which may not be consistent with a bioprinted pancreas.

Bibliography

[1] CCFA. "Clinical Trials 101." *CCFA*. Crohn's & Colitis Foundation of America, 1 June 2012. Web.15 Jan. 2014.

[2] Coatney, Sue, et al. "3D Bio-Printing." *Berkeley*. UC Regents, 17 Apr. 2013. Web. 10 Dec. 2013.

[3] Cowan, Patricia A., et al. "Pancreas Transplantation." *Medscape*. WebMD, n.d. Web. 10 Dec. 2013.

[4] Davis, Nicolynn E., et al. "Enhanced Function of Pancreatic Islets Co-encapsulated with ECM Proteins and Mesenchymal Stromal Cells in a Silk Hydrogel." *Biomaterials* 33.28 (2012): 6691-97. Web.

[5] Health Action International. “Life-saving Insulin Largely Unaffordable." *Haiweb*. HAI, 1 June. 2002. Web. 10 Dec. 2013.

[6] Hsu, Jeremy. "Tiny 3D-Printed Liver Slices Pave Way for Growing Organs." *LiveScience.com*. N.p., n.d. Web. 10 Dec. 2013.

[7] Leckart, Steven. "How 3D Printing Body Parts Will Revolutionize Medicine." 2*Popsci*. Popular Science, 6 Aug. 2013. Web. 10 Dec. 2013.

[8] Loffredo, Francesco, and Richard T. Lee. "Therapeutic Vasculogenesis: It Takes Two." *NLM*. U.S. National Library of Medicine, 18 July 2008. Web. 15 Jan. 2014.

[9] Organovo Holdings. “Bioprinting Advantages." *Organovo*. Organovo Holdings, n.d. Web. 10 Dec. 2013.

[10] John Hopkins University. "Pancreas Transplant." *John Hopkins Medicine*. John Hopkins University, n.d. Web. 10 Dec. 2013.

[11] Shimizu, Ippei, et al. "Excessive Cardiac Insulin Signaling Exacerbates Systolic Dysfunction Induced by Pressure Overload in Rodents." *Journal of Clinical Investigation* 120.5 (2010):Web.

[12] Vergani, Andrea, et al. "Effect of the Purinergic Inhibitor Oxidized ATP in a Model of Islet Allograft Rejection." *Diabetes* 62.5 (2013): 1665-75. Web.

[13] Wiki Author, “Biotechnology” *National Center for Biotechnology Information*. U.S. National Library of Medicine, n.d. Web. 11 Dec. 2013.

[14]"Pancreas Transplant." *MedlinePlus*. U.S National Library of Medicine, n.d. Web. 15 Jan. 2014.