Bioengineered Hearts

By: Rivky Loeb

Rivky graduated in June 2014 with a B.S. in biology.

Abstract

Heart disease is one of the highest causes for fatality in the world. Although many such diseases can be treated by a heart transplant, this in itself can cause countless problems. Aside from the high demand for donor hearts, there is the risk of the patient's immune system rejecting the transplanted heart. A bioengineered heart would reduce the need for donor hearts, and thus save countless lives. Finding a suitable scaffold, obtaining appropriate cells, and ensuring that the tissue will function properly are the main focuses in creating an artificial heart. While most of the studies done have been concentrated on creating cardiac tissue rather than the full organ, with the integration of these aspects scientists are getting closer to the goal of engineering a fully functioning artificial heart.

Introduction

Heart failure is one of the most prevalent causes of death in the world. There are many different diseases affecting the heart. For many of them, the only answer is a heart transplant. Although this has saved countless lives, transplants remain an imperfect solution. Firstly, the list of people requiring new hearts is a long one, but the amount of donor hearts available is few. Once a patient is approved for a heart transplant, s/he is added to a waiting list, which is part of a national allocation system run by the Organ Procurement and Transplantation Network (OPTN). There are approximately 3,500 people on the list, and waiting times can range from about six months to more than a year.

Even once a heart is found for the patient, and the transplant is successful, the patient is still not out of danger. Although the heart itself is healthy, it may not function properly in the recipient, causing a number of complications. One such concern is Primary Graft Dysfunction, which is the most frequent cause of death in the first 30 days after a transplant (National Institutes of Health, 2012).

According to the OPTN, the one year survival rate for patients in the U.S. aged 18-34 is almost 85%, with 75% 3 year survival rate, and 66% five year. The 1, 3 and 5 year survival rate for recipients age 35-50 is slightly higher, at 88%, 81% and 74% respectively (OPTN). Although these numbers are relatively high, one of the main causes for heart transplant failure in the first year after the transplant is due to the patient's immune system rejecting the foreign organ. To prevent this, immunosuppressant drugs are administered; however, this can cause damage to other organs such as the kidneys and liver. High cholesterol, diabetes and cancer are also some risks of the anti-rejection drugs (Bhimji, 2011). The gap between the supply and demand for donor organs, as well as the lifelong consequences for the patient, make the creation and implantation of a bioartificial heart a desirable alternative. While the construction of a functional whole organ has not yet been accomplished, tissue engineering and regenerative medicine research have obtained promising results for heart regeneration.

Bioengineers have been working on creating fully functioning organs that would eliminate the need for donors. This would solve the issue of patients not receiving a heart in time, in addition to getting rid of any concerns of rejection. There have been many studies done all in the hope of answering the question, is growing a new heart for a patient a foreseeable goal of the near future?

When trying to solve such a complicated issue there are numerous factors that must be taken into account, and many different angles that the problem can be studied through. With the case of the engineered heart, some of those factors include finding a suitable base, or scaffold for the organ, deciding what kind of cells would be appropriate for actually building the organ, and maturing the construct to develop some form of contractile and pump function.

Methods

The information in this paper was obtained by analysis of scientific articles and research papers. Various online databases and medical journals were used, accessed mostly via PubMed or the Touro College database. Most of the information is based on experiments done using rat cells since this is the most practical way of obtaining the large quantity of cells needed. Although the data is not completely applicable to human cardiac cells, researchers hope that these studies will provide insight into bioengineering of human hearts.

Discussion

The first major issue in organ engineering is creating a scaffold with enough elasticity, porosity, and strength to enable the cells to grow correctly, thus resulting in a functioning organ or tissue. The makeup of the scaffold is vital to the process because native tissues contain different cell types, with each cell type having its own unique three-dimensional (3-D) extracellular matrix environment, and mechanical properties. In addition, there are many other structures that must be taken into account, such as blood vessels. An ideal construct should display the functional and structural properties of natural heart muscle, and therefore should be contractile, vascularized, and electrophysiologically stable. To recreate such complexity in engineered tissues various approaches have been used.

Much of the research on cardiac tissue engineering that has been done was focused more on small sections of tissue than on the whole heart. However, this can be an important start to full organ engineering, as the results of such studies can be incorporated into studies dedicated to heart engineering.

Scientists have approached the issue of scaffolds from many different angles. In one such approach, cells are seeded on a degradable scaffold on which they reorganize into engineered tissues.

A group of bioengineers set out to research different designs for scaffolds to determine which would be most conducive to cardiac tissue growth. They were able to create two layer scaffolds, with fully interconnected pore networks, which aided in guiding the pattern of cell growth. The material used was a synthetic elastomer poly(glycerol sebacate), known as PGS. This polymer was chosen because of its ability to reproduce the mechanical stiffness and elasticity of the extracellular matrix. Additionally, PGS is supportive of blood vessel formation, and cardiogenesis. Different scaffolds were created with different properties, such as pore size, and thickness. Two layer scaffolds 200µm in total thickness, with interconnected pores were found to be the most effective for allowing heart cells to survive and form functional connections. PGS scaffolds provided a platform for patterned cell distribution while maintaining the geometric and mechanical properties of normal heart muscle (Neal, et al., 2013). A similar study done at Duke University used the flexible material PDMS (polyDimethylsiloxane) to create molds with elliptical pores. Such pores enabled the enhanced diffusion of oxygen and nutrients to the cells (Liau, et al., 2011).

In addition to the design for the scaffold, researchers must determine what kind of material should be used. One material, called Poly(N-isopropylacrylamide) or PNIPAAm, is a polymer recently emerging as a possibility due to its favorable properties. PNIPAAm has controllable features and switchable surface properties, making it an ideal option for scaffold formation. For example, PNIPAAm has a lower critical solution temperature (LCST) of 32oC. This is important because it will shrink and become hydrophobic at temperatures above the LCST, and hydrophilic at temperatures below. Whereas hydrophobic surfaces are attractive for cell attachment, detachment of cells and tissues from these templates requires either an enzymatic reaction or physical scraping, both of which can damage the cells. The use of PNIPAAm enabled controlled cell detachment by adjusting the temperature (Tekin, et al., 2011).

Another advantage to using PNIPAAm is its dynamic properties. Previously, micromolds were used to create microgels. However, these scaffolds had static structures, meaning the surface properties and patterns could not be changed after fabrication. PNIPAAm gel molds were able to be engineered to control 3-D organization of the cells by mimicking the complex native tissue architecture.

Additionally, the polymer is a hydrogel, which would enable blood vessels to form in the organ. Coating surfaces in PNIPAAm served to induce capillary network formation. This is especially important for tissues to function properly (Tekin, et al., 2011).

Recently, a group of scientists experimented with adding single-wall carbon nanotubes (SWCNTs) to PNIPAAm to improve the function of the base gel for use in myocardial repair. SWCNTs are sheets of graphene rolled into a seamless cylinder. They have remarkable electrical properties, which may even exceed the best metals or semiconductors known, as experiments have shown (McEuen, et al., 2002). They theorized that the SWCNTs would improve the bioactivites and adhesion of the hydrogel to the cells. After the cells were applied to the gel, it was observed that as expected, cell adhesion and proliferation was about 1.71 times greater in the PNIPAAm/SWCNT hydrogel than in the PNIPAAm. The results indicated that the incorporation of SWCNTs greatly enhanced the bioactivity of the PNIPAAm and aided in its functioning as a scaffold (Li, et al., 2014). Finding a suitable material for the scaffold is one main step in the process of tissue engineering.

Another option recently being explored for use as scaffolds, are decellularized organs. As mentioned previously, even if donor organs were not in short supply, the transplant recipient would still be at risk of immune rejections and lifelong immunosuppression treatment. To date, although numerous modern technologies have been employed to fabricate new tissues, the creation of a functioning whole organ is still in progress. The use of decellularized matrices would be a step in the right direction, as it would overcome the need for the bioengineer to artificially recreate the conditions for cell deposition. It would offer a microenvironment with preserved natural geometry and vascular networks, which would enable cells to grow in the correct patterns (Moroni , Mirabella, 2014).

In one such study, hearts were decellularized with detergents, which preserved the underlying ECM, providing acellular, perfusable vascular architecture, and intact chamber geometry. All cellular material which would induce an immune response, such as cardiac and smooth muscle cells, DNA, RNA and soluble proteins, were removed. This left behind only the collagen, laminins and other structural proteins as a scaffold. Fiber orientation and composition remained intact, as did the arterial and venous basement membranes. As shown in figure 1, the detergent SDS was successful in removing all cells, while the Triton detergent left cells behind (Ott, et al., 2008).

The scaffold or decellularized organ now must be seeded with cells. This leads to the next issue regarding tissue engineering: what kind of cells should be used. Many researchers in the field use a mixture of two or more cell types, such as endothelial precursor cells to line blood vessels, and muscle progenitors, which are
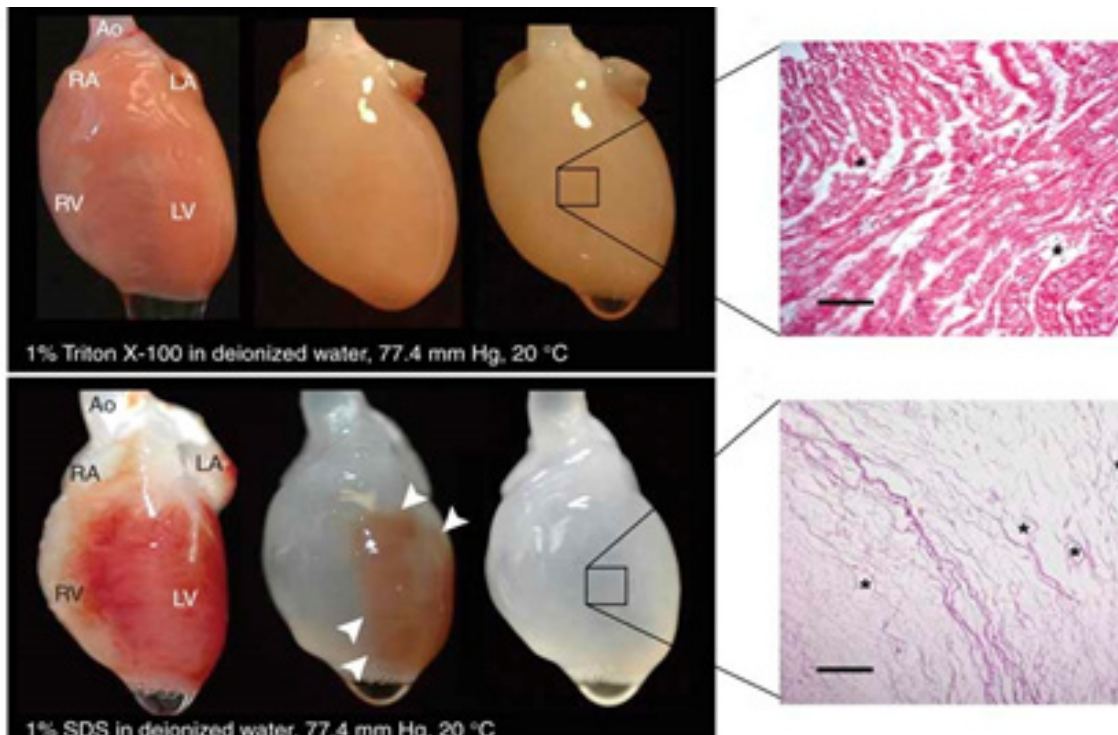
Figure 1:
Hearts decellularized using different detergents. Asterisks indicate intact vascular network.
Source: Ott HC, et al. 2008

similar to stem cells, to seed the walls of the chambers. These can be derived from induced pluripotent stem (iPS) cells- adult cells genetically reprogrammed to an embryonic stem cell-like state, which can be coaxed by scientists to become any kind of cell. This is useful because these can be taken from the patient, and used to make immunologically matched tissues (Maher, 2013). A team from the University of Pittsburgh recently used iPS cells generated from human skin cells to create precursor heart cells called MCPs. The cells were placed on a decellularized scaffold, and grew and developed into heart muscle. After 20 days, the tissue began showing cardiac muscle function (Discovery News, 2013).

Alternatively, as in a different study, heart cells were isolated from neonatal rats and digested in a trypsin solution until dissociated into a single cell suspension. Other steps were done to isolate the cells, and at the end of this method 63% cardiomyocytes, 33% cardiac fibroblasts, 3-4% smooth muscle cells and 2-3% endothelial cells were collected to be used to seed the scaffold (Neal, et at., 2013). Tissues constructed from these heart cell mixtures showed advanced structure, determining that creation of optimal cardiac tissue constructs depends on a mix of non-monocytes and cardiac monocytes (Zimmermann, et al., 2004).

A group of scientists using similar neonatal cells performed experiments to determine the effect of different factors on the growth of the cells in cultures and on the scaffold. For example, by analyzing the gas and CO2 levels in different vessels, they were able to determine what environment would be best for the cell growth. Additionally, by analyzing the density of the seeded cells, they determined that the minimal cell seeding density necessary to maintain construct structural integrity was achieved by using 1.4x106 cells per scaffold. For interconnected tissue structure, 8x106 cells per scaffold were required. From all the collected data, the researchers concluded that structural and functional properties of constructs were improved by seeding polymer scaffolds at high densities using rotating vessels. This study demonstrated that dissociated cells cultured on 3D scaffolds under favorable conditions were capable of forming engineered constructs with features resembling those of native tissues (Carrier, et al., 1998).

Other favorable properties for cell growth were determined in another study. Various factors were tested for their role in engineered heart tissue. High collagen content in the mixture yielded tissue with higher stiffness, but less contractile force development. Decreasing the collagen content yielded tissue with soft matrix structure, but improved contractile properties. Additionally, the inclusion of horse serum was found to be beneficial for engineered heart tissue development (Zimmermann, et al., 2004).

Once the scaffold is prepared and the cells cultured and seeded, the next step is to observe and determine whether the cells will behave as cardiac muscle cells. This includes being able to contract, and electrically conduct signals. While full engineered heart transplants have not been perfected, scientists have been successful on smaller scales. Biomedical engineers at Duke University were able to grow a three dimensional human heart muscle that acted like

natural tissue, in that it conducted electricity at about the same speed as natural heart cells, and contracted appropriately (Duke University, 2013). Using pluripotent embryonic stem cells permeated with fibroblasts, cardiac tissue was created with the capabilities of generating fast, uniform action potential propagation with velocities ranging from 17.8cm/s to 24.1 cm/s, and contractile force of 2mN (Liau, et al., 2011).

With the decellularization method, the recellularized hearts were placed in bioreactors that mimicked the sensation of beating. A combination of electrical signals was used to help synchronize the cardiocytes seeded on the scaffold, along with physical beating motions induced by a pump. After eight to ten days in the bioreactor the hearts were able to beat by themselves. However, when the team implanted the engineered hearts into rats, none of the hearts were able to perform the blood-pumping function at the degree that is required of the heart (Maher, 2013).

In a study done in Tokyo, layered cell sheets coated with PNIPAAm were created and implanted into the hearts of rats. At first the beating of engineered tissue, at 96bpm, was relatively slow in comparison to host hearts, 332bpm; after four weeks it had gotten stronger, though not as fast as the host tissue. A positive factor was that neovascularization occurred in the tissue, which aided it in functioning properly (Shimizu, et al., 2002).

In a different experiment, engineered heart tissue was created and implanted onto an infarcted area in rat hearts. When evaluated later, the engineered tissue showed electrical impulses in synch with the native tissue. It was also evident that the implanted tissue had prevented further dilation and induced wall thickening of infarcted myocardial segments. The epicardial activation of hearts with the tissue graft was normal, indicating undelayed coupling of the grafted tissue to the host tissue. The tissue was also able to propagate electrical potentials (Zimmermann, et al., 2006). This shows that tissue created in vitro can possibly function as natural cardiac tissue in vivo.

Conclusion

Although a completely functioning heart has not yet been engineered, scientists are headed in the right direction. Using scaffolds made of different materials and with different designs, they have been able to stimulate cell growth in the right patterns for tissue construction. Under the right conditions, the constructs behaved in a manner similar to native cardiac tissue. Such engineered tissues have even been shown to function almost as well as natural tissue in vivo. With the integration of these different aspects, and advances in bioengineering, scientists are getting closer to the goal of creating a fully functioning artificial heart.

References

Bhimji S. Heart transplant. Medline Plus. http://www.nlm.nih.gov/medlineplus/ency/article/003003.htm. Updated May 4, 2011.

Carrier RL, Papadaki M, Rupnick M, Schoen FJ, Bursac N, Langer R, Freed LE, Vunjak-Novakovic G. Cardiac Tissue Engineering: Cell Seeding, Cultivation Parameters, and Tissue Construct Characterization. Biotechnology and Bioengineering. Nov 1998; 64(5): 580-589.

Discovery News. Stem Cells Grow Beating Heart. Aug 2013. http://news.discovery.com/tech/biotechnology/stem-cells-grow-beating-heart-130814.htm

Duke University. Biomedical engineers grow 3D human heart muscle that acts just like natural tissue. News Medical. http://www.news-medical.net/news/20130507/Biomedical-engineers-grow-3D-human-heart-muscle-that-acts-just-like-natural-tissue.aspx. May 7, 2013.

Health Resources and Services Administration. Organ Procurement and Transplantation Network. http://optn.transplant.hrsa.gov/latestData/viewDataReports.asp.

Li X, Zhou J, Liu Z, Chen J, Lu S, Sun H, Li J, Lin Q, Yang B, Duan C, Xing M, Wang C. A PNIPAAm-based thermosensitive hydrogel containing SWCNTs for stem cell transplantation in myocardial repair. Bio Materials. July 2014; 35(22): 5679-5688.

Liau B, Christoforou N, Leong KW, Bursac N. Pluripotent stem cell-derived cardiac tissue patch with advanced structure and function. Biomaterials. Dec 2011; 32(35): 9180-9187.

Maher B. Tissue engineering: How to build a heart. Nature International Journal of Science. July 2013; 499(7456): 20-22.

McEuen PL, Fuhrer M, Park H. Single-Walled Carbon Nanotube Electronics. IEEE Transactions on Nanotechnology; 2002

Moroni F, Mirabella T. Decellularized matrices for cardiovascular tissue engineering. Am J Stem Cells. Mar 2014; 3(1): 1-20

National Institutes of Health. What are the risks of a Heart Transplant. National Heart Lung and Blood Institute. http://www.nhlbi.nih.gov/health/health-topics/topics/ht/risks.html. January 3, 2012.

Neal RA, Jean A, Park H, Wu PB, Hsiao J, Engelmayr GC Jr, Langer R, Freed LE. Three-dimensional elastomeric scaffolds designed with cardiac-mimetic structural and mechanical features. Tissue Eng Part A. Mar 2013; 19(5-6): 793-807.

Ott HC, Matthiesen TS, Goh SK, Black LD, Kren SM, Netoff TI, Taylor DA. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. Nature Medicine. Jan 2008; 14: (213-221).

Shimizu T, Yamato M, Isoi Y, Akutsu T, Setomaru T, Abe K, Kikuchi A, Umezu M, Okano T. Fabrication of Pulsatile Cardiac Tissue Grafts Using a Novel 3-Dimensional Cell Sheet Manipulation Technique and Temperature-Responsive Cell Culture Surfaces. Circulation Research. Jan 2002; 90:e40-e48

Tekin H, Sanchez JG, Tsinman T, Langer R, Khademhosseini A. Thermoresponsive Platforms for Tissue Engineering and Regenerative Medicine. AIChE J. Dec 2011; 57(12): 3249-3258.

Zimmermann WH, Melnychenko I, Eschenhagen T. Engineered heart tissue for regeneration of diseased hearts. Biomaterials. April 2004; 25(9): 1639-1647.

Zimmermann WH, Melnychenko I, Wasmeier G, Didie M, Naito H, Nixdorff U, Hess A, Budinsky L, Brune K, Michaelis B, Dhein S, Schwoerer A, Ehmke H, Eschenhagen T. Engineered heart tissue grafts improve systolic and diastolic function in infarcted rat hearts. Nature Medicine. April 2006; 12(4): 452-458.