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PRIMER

The importance of the extracellular matrix: a primer for "perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart"

Bethanie Borg

Abstract

Heart disease is the leading cause of death in the United States. The number of donor organs is fewer than the patients on the transplant waiting list, while immunosuppression and rejection are common amongst those who receive a transplant. In an article entitled "Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart" by Harald Ott et al (2008), a major step was taken on the road to developing an alternative to donor organ transplantation. Rat hearts were decellularized with perfusion using the detergent sodium dodecyl sulfate (SDS), leaving the extracellular matrix intact. Heart constructs were recellularized in a perfusion bioreactor with neonatal cardiac cells. After 8 days, the constructs were able to respond to electrical stimulation and beat synchronously. Although the heart was not able to beat at the same level needed in order to sustain life, this was the first successful attempt at creating a fully functional bioartificial heart.

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Purpose: To unpack the complicated methods used by Ott et al. in order to fully understand the importance in the progression of creating a functional bioartificial heart that could possibly replace the need for donor-organ transplants.

Introduction

Heart disease is the number one cause of death in the United States. While heart transplantation is the current solution for end-stage heart failure, the search for a contemporary solution for heart failure is needed in the biotechnological field. A new method for creating bioartifical hearts is described in the paper "Perfusion-decellularized matrix: Using nature's platform to engineer a bioartifical heart," conducted by Harald Ott, Thomas Matthiesen, Saik-Kia Goh, Lauren Black, Stefan Kren, Theoden Netoff, and Doris Taylor. This article has been cited as one of the first successful bioartifical heart experiments that have resulted in the contraction and beating of the recellularized heart. The purpose of this primer is to unpack the complicated methods used by Ott et *al* in order to fully understand the importance in the progression of creating a functional bioartificial heart that could possibly replace the need for donor-organ transplants in the future.

Organ transplant and problems created after surgery: the need for a new treatment for heart disease

There are more than 5 million people in the United States who suffer from heart disease,

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and it is the number one cause of death (Song and Ott 2011). Currently, the leading treatment for end stage heart failure is heart transplantation (Eitan et al. 2010; Ott et al. 2008). Though organ transplantation undoubtedly has had its success, there are many problems that can arise.

The largest problem is the lack of donor organs for waiting individuals. In the United States alone, there are 3,000 patients in need of a heart transplant, with only around 2,000 donor hearts available (Ott et al. 2008). Patients can wait months on the transplant list before receiving a donor heart depending on the severity of their condition and if their blood type matches the donor. It is important for a solution to this problem to be solved as many people on the transplant list die before ever getting a donor heart.

Immunosuppression
Partial or complete suppression of the immune response of an individual.

Immunodeficiency
Inefficiency of the immune system to protect and fight off infections within the body.

In addition, problems that can arise after transplantation are **immunosuppression** and **immunodeficiency**. In order for a donor organ to be accepted in the new host, the donor and the host must have the same blood type and antigens. If they do not match, there is a significant risk of rejection due to the host's immune system recognizing the donor organ as an invader. As a result, the immune system will attack the donor organ and kill it, and the patient will need to be placed on the transplant list again (Arkwright et al. 2002). In order to lessen the risk of rejection, immunosuppressants are taken daily for the rest of the patient's life. Suppression of the immune system can lead to secondary diseases, however, such as diabetes, kidney failure and hypertension (Mirmalek-Sani et al. 2013). This also increases the risk of infection by bacteria and other microorganisms. A functional bioartificial heart may have the capability of avoiding these problems because it could help with the lack of donor organs for transplant and patients would not have to be on immunosuppressants

An intact extracellular matrix is essential for the creation of a bioartificial heart

One critical piece in making a bioartificial heart is an intact heart extracellular matrix (ECM). The extracellular matrix is a network of proteins important in the beginning of early embryology to form the framework of each organ (Badylak et al. 2009). In adults, the ECM composition varies widely among different organs as to cater to their unique functions and plays a role in structural support of the organ, proliferation and migration of cells, and cell-cell interactions of all organs (Bowers et al. 2010).

Within the heart, the ECM is composed of a number of different proteins that interlock to create a strong scaffold. Collagen I and III, along with other collagen types, work together to provide structural support to the ECM. Fibronectin is a glycoprotein that binds collagen proteins of the cell that assists cells in moving through the ECM. This facilitates the passing of cells or molecules through the ECM into the intracellular or extracellular environment. Laminin is a protein that is important to the basal lamina, which is a part of the ECM where epithelial cells rest. Laminin gives the ability of the ECM to resist tensile strength forced on the basal membranes of cells, such as the tensile strength of a heartbeat (LaBleu et al. 2007). Together these proteins provide structural support, tensile resistance, and transport to the ECM, all which are important for proper heart function.

An important feature of ECM proteins is that they are among the most conserved proteins among vertebrate species (Song and Ott 2011). These proteins are consistently present within the ECM and have little immunogenic qualities. In other words, the decellularized scaffold, consisting of only ECM proteins, should not trigger a response by the host immune

system when transplanted into the host. This was confirmed in a recent experiment that transplanted a newly decellularized rat liver scaffold into another rat for 28 days (Mirmalek-Sani et al. 2013). Host cells populated the scaffold throughout the 28 days and did not lead to an increase in white blood cells or macrophage precursor cells, which would increase during an immunological response (Mirmalek-Sani et al. 2013). Even more impressive was that there was no immune response to a pig liver construct that was transplanted into a rat host. This indicates that the ECM proteins between the two species were similar enough to avoid an immunological response by the host (Mirmalek-Sani et al. 2013). The results from the experiment by Mirmalek-Sani et al. can be interpreted to suggest that ECM from other animals, such as a pig heart ECM, could be used as a scaffold for human transplantation as long as the scaffold is similarly sized to a human heart and the ECM proteins are similar enough to avoid an immunological response and prevent organ rejection (Mirmalek-Sani et al. 2013).

Successful decellularization involves minimal damage to the ECM

Decellularization is the process of removing all of the DNA and cells from an organ while keeping the extracellular matrix intact. The common methods of decellularization include physical, chemical, and enzymatic techniques (Gilbert 2012). There is rarely only one technique used to completely decellularize a tissue, but the combination of techniques is essential when determining which is best in the respect of leaving the ECM intact.

There have been many methods that have attempted to successfully decellularize hearts, but they have resulted in damage to the extracellular matrix. In an earlier study by Ott and colleagues, a method of decellularization called immersion decellularization was used. In immersion decellularization, the whole organ is completely immersed in a solution containing a harsh detergent. The detergent destroys the cells on the outside of the organ first and reaches the inner cells through diffusion. The detergent cannot reach the inner cells before they start to release proteases, which destroy proteins in the organ, including the extracellular matrix ("Perfusion Decellularization"). In contrast to immersion decellularization, perfusion decellularization continuously circulates a solution throughout the organ by using its natural vasculature. This allows the inner and outer cells to undergo decellularization at the same time and decreases the probability of proteases to be released. This method of perfusion decellularization was used in the Ott et al. (2008) paper and was key to complete decellularization of the heart without damaging the ECM.

Recellularization of the decellularized construct is important for correct function of the heart

After decelluarization of the heart, the next step in creating a bioartificial heart is to recellularize the **construct** with cardiac cells. The goal of Ott et al. (2008) was to recellularize the construct in a perfusion recellularization bioreactor with the correct cell types and allow the cells to grow to a thickness that could support heart contraction. Before perfusion recellularization methods were introduced, the typical method of recellularization used two-dimensional non-perfused tissue culture dishes to grow the cells. The major problem with the non-perfusion recellularization method is that the **myofibers** do not grow to a thickness that can support contractions that can move blood throughout the whole body

Construct
Another term for the decellularized heart.

Myofibers
A muscle cell forms a large muscle when grouped together.

of an animal (Ott et al. 2008). Perfusion recellularization differs from non-perfusion decellularization in that the solution containing nutrients is continuously cycled throughout the bioreactor. This allows for the solution to have consistent nutrient supply while also removing wastes that could affect the rate of growth of the cells and presence of proteins within the cells (Ott et al. 2008).

Actin and myosin work together to contract the heart

In order for the heart to beat, the heart must contract. Two major proteins, actin and myosin, must work together in order for muscle to contract. Myosin plays a role in cardiac velocity output and force, while actin is required for normal contractility of the heart (Ilkovoski et al. 2005). In a resting state, a protein called tropomyosin is bound to actin and prevents myosin from binding to the actin. As the **sarcoplasmic reticulum** within the muscle is depolarized, it releases calcium ions. Troponin then binds the calcium ions and releases the bond between tropomyosin and actin, exposing sites to which myosin can bind. This interaction between myosin and actin results in muscle contraction. If these proteins are not present within the heart muscle, the heart will be unable to contract and cannot pump blood throughout the body. Presence of these proteins in a bioartificial heart will play a major role in heart contraction.

Sarcoplasmic reticulum
An organelle found in muscle cells that release calcium when depolarized, which allows the muscle cell to contract.

Another important protein involved in cardiac muscle contraction is connexin-43. Connexin-43 is a transmembrane protein that forms gap junctions linking the membranes of adjacent cells. These gap junctions allow the simultaneous coordinated depolarization of cardiac muscle cells, resulting in a heartbeat (Eckardt et al. 2004). Without these gap junctions, the cardiac cells would not depolarize at the same time and would not be sufficient to contract the heart and support a living organism.

Creating the first bioartificial heart: Advancements made by Ott et al *(2008)*

Perfusion decellularization

Importance of decellularization to the biomedical field

Ott et *al* provided the scientific community with a method that allowed for complete decellularization of a whole heart with no damage to the ECM. Perfusion decellularization with SDS gently washed the cells and intracellular proteins out of the heart while leaving the important proteins of the ECM intact. The ECM is important for structural integrity and vasculature of the heart, as well as controlling mechanical properties. The ability for this method to leave coronary vasculature and the ECM intact is the first step in the pathway of creating a bioartificial heart.

Perfusion decellularization with sodium-dodecyl sulfate resulted in a completely decellularized heart

The goal of Ott et al. (2008) was to create a completely decellularized heart without damaging the ECM. The creation of a completely decellularized heart (construct) is important in order to successfully reseed the construct with new cells. Remaining DNA and intracellular proteins can interfere with the success of recellularization and can also lead to an immune response when transplanted into a host. Ott et *al* used the method of perfusion decellularization to successfully remove cells and DNA from whole rat hearts (Ott et al. 2008).

Perfusion decellularization continuously circulates a perfusate throughout the organ in a device called a Langendorff Apparatus. The Langendorff Apparatus creates a flow of perfusate throughout the heart vasculature in

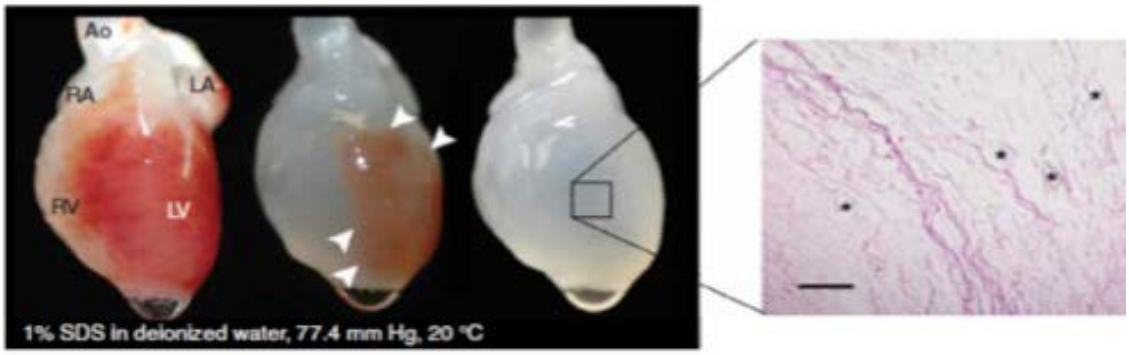

Figure 1. **"Visualization of a completely decellularized rat heart using 1% SDS."** The arrows surround the left ventricle, which is the last part of the heart to become fully decellularized. Ao = aorta; RA = right atrium; RV = right ventricle; LA = left atrium; LV = left ventricle." This image is important in that it shows that SDS was able to completely remove cells from the heart, leaving a translucent appearance. Evidence of vasculature is indicated by *. Adapted from Ott et al., 2008.

order to prevent unequal decellularization and release of proteases. The heart is attached to the chamber through a tube inserted into the aorta. The perfusate passes through this tube into the heart and through the vasculature. The perfusate then circulates back to the chamber and is re-warmed to 37°C, or normal body temperature. As optimum heart function is obtained at normal body temperature, it is important for the system to remain at a constant temperature because heart rate and contractile force are influenced by changes in temperature (Skrzypiec-Spring et al. 2007).

The type of perfusate used is important because harsh chemicals can damage the ECM and other chemicals may not effectively completely decellularize the heart (Gilbert et al. 2006). In a comparison of one percent solutions of three different detergents (polyethylene glycol, Triton-X100, and **sodium-dodecyl sulfate** (SDS)), Ott et al. (2008) found that SDS was the only solution that completely decellularized the heart. The SDS was continuously circulated through **antegrade coronary perfusion** in the Langendorff Apparatus in order for the ECM to remain undamaged. One indication that the heart had been completely decellularized was the translucent appearance of the construct (Figure 1). The translucent appearance indicated the absence of cardiac cells and endothelial cells that are found in the heart. The last portion of the heart to become translucent was the left ventricle because it consisted of thicker myocardium than the rest of the heart.

In addition to the absence of cardiac and endothelial cells, nuclei and DNA were also absent (Figure 2). To confirm the absence of nuclei and DNA, cadaver hearts (used as a control) and decellularized constructs were compared through fluorescent microscopy **histology** with **diamidino-2-phenylindole (DAPI)**, which causes nuclei to fluoresce blue. The absence of blue fluorescence in the decellularized construct in Figure 2 correlates with the absence of nuclei within the construct. Absence of nuclei and DNA is important in order to successfully recellularize the construct. Failure to fully remove the nuclei and DNA could result in stunted growth of the cardiac cells used to reseed the construct and would result in a heart that is not strong enough to beat on its own.

Decellularization yielded a construct with an intact ECM and vasculature

Important ECM proteins, including collagen I, collagen III, laminin, and fibronectin, were present within the decellularized ECM (Figure 2). It is evident that the ECM remained undamaged because the coronary vasculature of the heart also remained intact. Vessels of varying sizes remained recognizable and

Sodium-dodecyl sulfate (SDS)
An ionic detergent that lyses cells and unravels proteins through interrupting covalent bonds.

Antegrade coronary perfusion
A decellularization method that uses the natural direction flow of coronary vessels to carry the detergent solution throughout the heart.

Histology
The study of cells performed by examining the content and structure of the cells under a microscope. Cells are commonly stained with different dyes marking different structures and proteins within the cell.

Diamidino-2-phenylindole (DAPI)
A fluorescent blue dye that binds to the AT rich minor grooves in DNA.

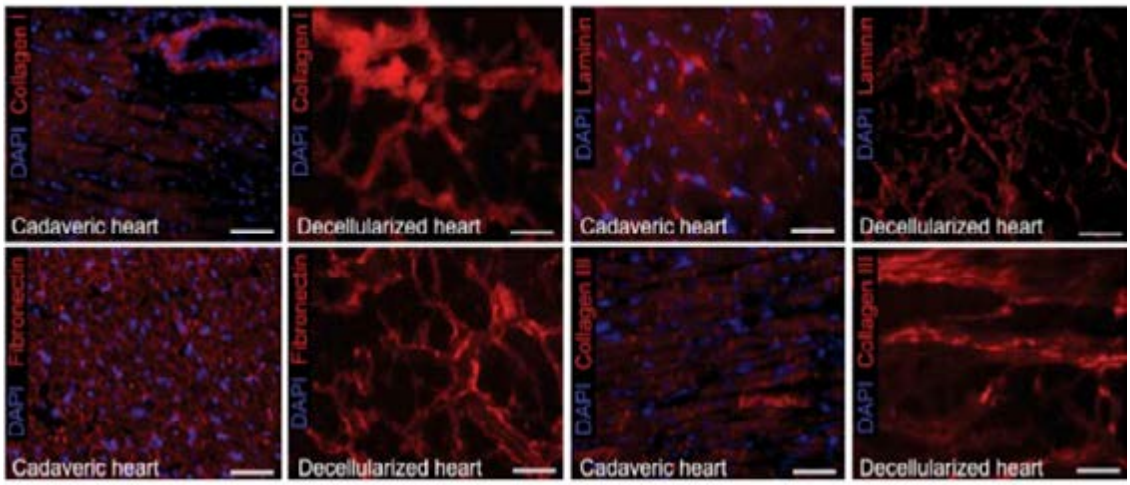

Figure 2. "Depletion of nuclei in decellularized heart constructs after perfusion with SDS." DAPI stains nuclei blue, while protein is stained in red. Collagen, laminin, and fibronectin from the extracellular matrix are preserved in the decellularized heart while the absence of DAPI indicates the absence of nuclei." It is important for DAPI to be absent since the nuclei need to be removed during decellularization. Presence of collagen, laminin, and fibronectin indicate that extracellular matrix proteins are still present after decellularization. Adapted from Ott et al, 2008.

intact, such as large arteries and veins (Figure 3a). In order to test the permeability of the ECM of the vasculature, Ott et *al* secured the decellularized construct to a host aorta from a living rat (Ott et al. 2008). After releasing the clamp, the blood flowed through the construct's arteries without leakage through the ECM, confirming that the arteries and veins remained intact (Figure 3b). The ability of Ott et al. (2008) to keep artery and vein ECM intact after decellularization was not only important in order to successfully recellularize the construct, but also in order for the heart to be able to circulate blood after recellularization.

Successful Recellularization of Whole-hearts

Importance of recellularization in the biomedical field

The recellularization perfusion method used by Ott et al. was an important advance in the biomedical field because it caused cardiac muscle to grow to a viable thickness that supported blood flow. The use of a bioreactor gave the heart precursor cells continuous nutrition in order to grow and replicate efficiently. Following recellularization, important proteins needed for cardiac depolarization and muscular contraction were also found in the left ventricle, where the heart construct was injected with cells. Without these proteins, the heart construct would not be able to beat and would not support a living organism. This recellularization perfusion method is an important second step in creating a fully functional bioartificial heart.

Recellularization via injection

Before recellularization, the decellularized constructs were placed in a bioreactor specific for whole-heart organ culture and were perfused with phosphate buffered saline (PBS) for 124 hours (Ott et al. 2008). PBS was used to completely rinse away any SDS solution that had been used to decellularize the heart. If any SDS was left in the construct while recellularizing the heart, the SDS could lyse the new cells and cause incomplete recellularization of the construct. The constructs were then perfused with oxygenated cell medium for 24 hours. The media contained nutrients essential for cell growth and those nutrients would be

present in the ECM when the constructs were recellularized.

Decellularized constructs were recellularized with media containing PBS, **cardiomyocytes, fibrocytes, endothelial cells,** and **smooth muscle cells** (Ott et al. 2008). The cell media was injected into the left ventricle of the construct while in the bioreactor, and the construct was continuously perfused through coronary vessels with oxygenated cell medium for the duration of cell growth. Continuous perfusion is important in order for the cells to grow optimally and replicate efficiently. After 24 hours of perfusion, electrical stimulation was applied to the recellularized construct. The purpose of this stimulation was to create synchronization between all of the cells in the construct. Synchronization between cells is important because mature cardiac cells need to **depolarize** together in order for the heart cells to beat together and form a heart beat.

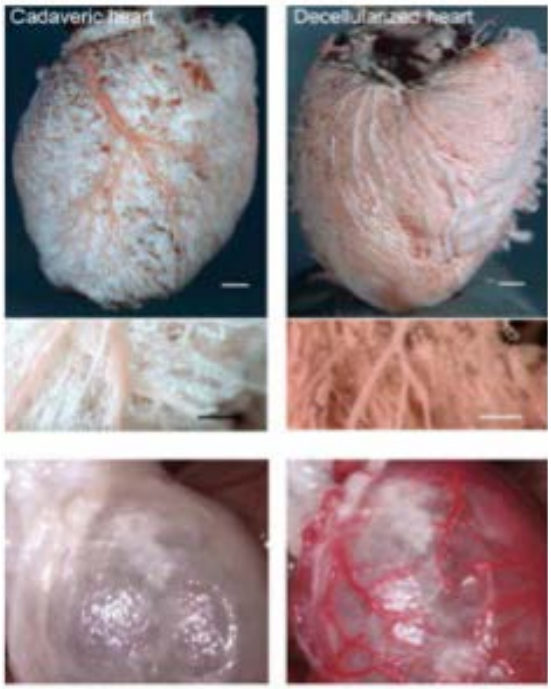

Figure 3. "Vasculature of a decellularized heart." (a) Coronary corrosion cast of a cadaver heart (left) and decellularized whole heart (right). (b) Visualization of the coronary arteries of a decellularized heart (left) through attachment to a host aorta (right)." Notice the arteries remain intact and do not leak after decellularization. This is an important indicator that the extracellular matrix has remained intact. Adapted from Ott et al. 2008.

Perfusion recellularization in a bioreactor allowed cardiac precursor cells to grow to a viable thickness

The perfusion recellularization methods used by Ott et al. to recellularize the decellularized constructs are significant because the constant perfusion in a bioreactor allowed the growth of viable cardiac muscle. Perfusion recellularization for eight days allowed myofibers to grow to a diameter ranging from 250 micrometers up to 1.1 millimeters (as compared to less than 50 micrometers in non-perfused constructs). The areas of the recellularized construct that had the greatest thickness of cellularity occurred at the sites of injection of the cells in the left ventricle.

As growth of cells within the construct is important, it is also important that the right proteins are present within the cells in order to make the construct contract. Major proteins that are involved in heart contraction are actin, myosin, and connexin-43. Histological analysis of cells within the left ventricle of the construct revealed the presence of cardiac myosin heavy chain, sarcomeric alpha-actinin, and connexin-43. Cardiac myosin heavy chain is known as "the molecular motor of muscle" and is needed in order to bind to actin and result in muscle contraction (Nakao et al. 1997). The presence of connexin-43 also signifies that the construct has the ability to spread the action potential to all cells to make the construct beat. The presence of these three proteins is important for depolarization and contraction of the cardiac muscle.

Cardiomyocytes Bundles
of muscle that form cardiac muscle.

Fibrocytes
An inactivbe cell that lacks biochemical activity and has the capability of differentiating into a fibroblast. Fibroblasts are connective tissue cells that can make collagen, a structural protein.

Endothelial cells
Cells that form a thin layer on the inner surface of a blood vessel, creating a boundary betwen circulating blood and the vessel wall.

Smooth Muscle Cells
non-striated muscle cells that are innervated by action potential from the autonomic nervous system. Gap junctions within these cells allow the action potential to be spread from one cell to all neighboring cells to result in simultaneous contraction.

Depolarization
Process in which the membrane potential of a cell changes immensely for a short time due to an influx of sodium ions, causing an action potential to spread across the heart. Depolarization of cardiac cells.

Electrical stimulation of whole-hearts

Importance of whole-heart experiments to the biomedical field

Pulsatile preload (diastole)
Initial stretching of cardiomyocytes prior to contraction.

Compliant afterload (systole)
Tension developed in the wall of the left ventricle duringejection into the aorta.

The whole-heart experiments by Ott et al. (2008) are significant because a beating heart was created. The pulsatile antegrade perfusion and coronary perfusion methods used were able to successfully recellularize the heart by distributing sufficient oxygen and nutrient levels to the cardiomyocytes and endothelial cells. After only 8 days of perfusion in the bioreactor, the heart was able to beat after electrical stimulation and was able to move fluid through the left ventricle and ascending aorta without the bioreactor pump. This level of contraction in previously decellularized whole hearts had not been reported before the experiment by Ott et al. (2008), and is the third major step in creating a functional bioartificial heart.

Systolic and diastolic flow simulation within the bioreactor were used to simulate heart flow

Epicardial
On the surface of the heart.

Proper solution flow throughout the recellularized heart was done through a flow pump that imitated systole and diastole events. Systole and diastole are the measurements of blood pressure while leaving the atrium and the ventricle. Diastole occurs during the beginning of the cardiac cycle, when blood leaves the atria and the ventricles are filled with blood. This measures the minimum pressure in the arteries when the heart is at rest. Systole measures the peak pressure in the arteries when the ventricles contract, sending blood out of the heart. The bioreactor conditions used by Ott et al. (2008) simulated diastolic and systolic flow throughout the heart construct. This simulation was achieved with both pulsatile antegrade perfusion and coronary perfusion.

In order for the cells within the construct to grow properly, two tubes were inserted into the heart to administer the oxygenated medium. The first tube was inserted into the left atrium. The oxygenated medium entered through the left atrium and into the left ventricle, simulating **pulsatile preload** (diastole). The medium then exited through the ascending aorta, which was where the second tube was inserted, simulating **compliant afterload** (systole). The pressure of the medium was adjusted in order to allow for the closure of the aortic valve between each pulse, just like a normal beating heart (Ott et al. 2008). The coronary arteries supply oxygenated blood to the heart itself. The compliance loop from the ascending aorta carried the oxygenated medium to the coronary arteries, which supplied the cardiomyocytes with oxygen and nutrients in order to grow and mature. The medium exited the coronary arteries through the right atrium. Electrical stimulation of the heart construct occurred through **epicardial** leads, allowing the construct to contract. The heart constructs were perfused for 8-28 days (Ott et al. 2008).

Recellularized constructs responded positively to electrical stimulation

The goal of recellularization by Ott et al. was not only to seed the heart construct with cardiac cells, but also to grow those cells to a thickness that could respond to electrical stimulation and make the heart beat. The frequency of electrical stimulation applied to the construct was an important factor in the ability for the construct to contract. The contractile force remained constant when there were less than 4 electrical stimulations per second (4 Hz). This relates to about 2 percent of adult rat heart function and 25 percent of a 16-week-old human heart function. If there was more than 4 Hz applied to the heart, there was a decrease in contractile

force. At 4 Hz, the heart construct was able contract in a normal fashion, including repolarization of the heart. Repolarization is the restoration of the membrane potential between the outside of the cell and inside of the cell. The resting potential of the cell is a negative value, and when it is excited, or depolarized, a heart beat results. Repolarization of the heart construct is essential in order for the heart to become depolarized again and continue a normal heartbeart.

Throughout perfusion, the response of the heart was measured with an electrocardiogram (ECG), the afterload pressure, and the left ventricular pressure (LVP). The heart construct was stimulated with 100 volts of electricity and the pump that was pulsing the oxygenated medium through the heart was off. On day zero, electrical stimulation of the acellularized heart resulted in an abnormal ECG with only one peak from the stimulation. The afterload pressure and LVP were nonexistent, indicating that the construct was not able to beat. By day 8, the heart was recellularized and important proteins, such as myosin and actin, were present in the cells. Electrical stimulation resulted in an increased afterload pressure and LVP, suggesting that the heart construct was able to successfully pump medium from the ventricle to the aorta (Ott et al. 2008).

The level of contraction of heart constructs presented by Ott et al. (2008) has not been reported previously in other experiments. After only 8 days of perfusion in the bioreactor, proteins important to heart depolarization and contraction were found throughout the left ventricle. The presence of these proteins allowed the heart construct to beat after electrical stimulation and the heart construct was able to move fluid through the left ventricle and ascending aorta without the bioreactor pump.

Conclusion

The experiments performed by Ott et al. (2008) were the first successful attempts at creating a fully functional bioartificial heart. Whole cadaver rat hearts were successfully decellularized without damaging the ECM, which had not been reported before this research. The decellularized construct was successfully reseeded through injection within a closed bioreactor system, which allowed for continual oxygen and nutrients to flow throughout the construct. The perfusion decellularization and recellularization methods both gave a more complete protocol in the process of creating a fully functional bioartificial heart. Perfusion in the bioreactor allowed the cardiac muscle to grow to a viable thickness that was able to support muscle contractions. Although the heart was not able to beat at the same level needed in order to sustain life, the heart was able to pump fluid on its own, which is a great advancement in the creation of a bioartificial heart. With continued experimentation, these bioartificial hearts may have the capability of replacing donor organ transplantation, easing the need for heart donors and allowing more end-stage heart disease patients a better opportunity in receiving a transplant.

Advancements in bioartifical hearts after the research by Ott et al. (2008)

There has been little advancement on the road to creating a fully functional bioartificial heart since the research done by Ott et al. was published. Many researchers continue to experiment with perfusion decellularization and recellularization protocols. Successful decellularization of heart constructs has been reported in numerous studies (Lu et al. 2013; Wainwright et al. 2010; Weyman et al. 2013). The problem limiting the progress of bioartificial hearts seems to be with recellularization of the constructs

and the ability of the construct cells to beat synchronously. One study tried to repopulate the construct with human induced pluripotent stem cells of cardiomyocytes, but the construct cells were not able to synchronously beat to push fluid through the vasculature (Lu et al. 2013).

There have been advancements, however, in regards to the problems of immunogenic reactions and organ rejection. One important study looked at the immunogenic qualities of natural ECM that was transplanted into both **allogeneic** and **xenogeneic** recipients. The study found that there was no immunogenic reaction by the host immune system after 28 days in either the allogeneic (rat to rat) recipient or the xenogeneic (pig to rat) recipient (Mirmalek-Sani et al. 2013). This indicates that the ECM proteins of both species were similar enough to avoid the trigger of the host immune system, and suggests that heart ECM of different animals could be used as a scaffold for human transplantation if they are similar enough in protein composition and size. This also implies that different sections of the heart ECM from animals, such as valves, could be used to treat human heart disease. Further research into both bioartificial whole hearts and heart sections is needed before use in clinical research.

Allogeneic transplantation
Transplatation of an organ into the same species from which that organ was taken from. For example, transplanting a rat heart into another rat.

Xenogeneic transplantation
transplantation of an organ from one species into a different species. For example, transplanting a pig heart into a human being.

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