Tissue Engineering and the Future of Transplant Medicine

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Abstract

Tissue engineering is an emerging field in medicine. The purpose is to synthesize new tissue that the body will not reject but will use to heal the body from its own cells. It will lay down new material, with hopes that the new tissue will develop and continue to regenerate. This is performed with the use of stem cells, scaffolding and growth materials. Tissue engineering was birthed as a solution for organ transplants. The technique for transplanting organs has a long history as it has evolved over the years and continues to progress with technology. There have been many advantages to transplants, however, there are still many complications. The future of tissue engineering is to completely eliminate any need for the transplant list because researchers will be able to develop full organs from a person's own cells that the body will not reject. Plastic surgery, skin grafts, and potential development of organs are just some examples of the areas in which tissue engineering has incredible benefits.

Tissue Engineering and the Future of Organ Production from Stem Cells

Introduction

Medicine has been available since ancient times; however, the methods and procedures doctors use to treat their patients have drastically changed from the early civilization times (Guthrie et al., 2020). Modern technology has advanced medicine in ways that were not thought possible. Through the development of technology over the years, the world has drastically changed for the better. Now, with modern-day technology, doctors are able to more efficiently and safely perform procedures such as heart transplants and brain surgery. An emerging trend in transplant medicine is tissue engineering. It has rapidly gained momentum in medical research due to the possibilities of healing and hope for patients. Tissue engineering has quickly become the future for transplants, wound healing, and other forms of regenerative medicine through the production of new tissue to heal the body from the inside out.

Organ Transplant

History of Organ Transplant

The process and discovery of organ transplant was a huge leap for the world of medicine. Organ transplant can be dated back to the time of ancient Greece. There are several manuscripts and Greek myths that involve the topic of transplanting, specifically relating to the body. Many scholars speculate that organ transplant or preservation in ancient history was the original basis for the current technique. Not only does it appear in Greek history, but many Roman and Chinese myths from ancient manuscripts hold extravagant tales concerning the removal of organs (Hamilton, 2012). These documents portray the transplant of organs in a deistic light because it was considered a sacred task, and often only performed for their gods. The reason for these

transplants stems back to the belief from these cultures that the preservation of a person's body after death, will allow them to have an exceptional experience in the afterlife. However, many of these stories are just myths or wives' tale and not true accounts of transplants. Nevertheless, historians have found accounts which hold evidence of Native Americans performing skin grafts in order to heal wounds, specifically burns.

During the 1600s, a man named Gasparo Tagliacozzi was one of the first plastic surgeons, but he used skin from different areas of the person's body to graft it into the reconstruction of facial features (Frank & Frank, 1918). He discovered that the patient's body would accept their own skin best because samples from another donor often caused an immune response which resulted in rejection of the transplanted skin (Frank & Frank, 1918). This was a huge discovery which is now understood as transplant rejection. The idea of using organs from other sources progressed to the point that in the early 1900s, doctors attempted to save several patients with renal failure by transplanting organs from animals such as pigs or goats into the patient's body (Barker & Markmann, 2013). They had successfully performed dozens of organ transplants between animals and decided to progress to humans. As one would imagine, the transplants in humans were unsuccessful, but it continued to spark new ideas and inventions in the minds of many aspiring medical pioneers and researchers.

In 1912, Alexis Carrell, a French surgeon, was awarded the Nobel Prize for discovering techniques to successfully reconnect blood vessels, specifically in the context of organ transplant surgeries (Barker & Markmann, 2013). Carell, with the help of a colleague, invented a machine that was able to keep the organs that were taken outside of the body healthy and allow them to be transplanted into another being (Barker & Markmann, 2013). Later on, this machine served as

the building block for successfully being able to preserve organs for extended periods of time to allow for the transportation of the organ to neighboring clinics or hospitals. The first successful transplant occurred in the 1950's in the United States with a team of surgeons. The patient had severe kidney disease with no hope of surviving without a new kidney. A group of doctors from a hospital in Boston were willing to try a transplant of a kidney from his twin brother. The surgery was incredibly successful with no transplant rejection of the new kidney. At the time, they were not completely aware that the reason for the success was due to the kidney being from his twin brother (Barker & Markmann, 2013). Tissue transplant had been around for several decades, but this was the first time that the patient's body did not reject the organ or cause an immune response (Barker & Markmann, 2013).

Following the success with the transplant of the twin brothers, many researchers were beginning to understand that the rejection of the organs was more importantly correlated to a person's immune system attacking the new organ. With this understanding, medical professionals started looking into ways to suppress the immune system for a time while the organ was transplanted so it would not be rejected (Barker & Markmann, 2013). Immunosuppressive therapies and medications were an emerging field at this time because there had not been a need for them prior to transplants (Colaneri, 2014). An immunosuppressive drug called cyclosporine was used with renal transplants and increased the success rate to roughly eighty percent (Colaneri, 2014). This was a significant advancement for transplants as researchers were now able to not only successfully perform the surgery but also see promising results with the patients. The mortality rate decreased significantly for patients needing transplants and the next several years consisted of an explosion of successful transplants moving from nonrenal organs to the

liver, pancreas and even heart (Barker & Markmann, 2013). The continued development of immunosuppressive therapies allowed surgeons to progress with organ transplants and see more positive outcomes. With the help of new developments, organ transplant has been advanced and altered with the emergence of new technology and discoveries every year.

Development of Modern-Day Organ Transplant

The process of organ transplant has been altered throughout the years, specifically with immunosuppressive therapies and new techniques. When a patient who is near death has been identified as a potential organ donor, the process begins with finding a match for their organs. The process of matching organs begins by having a medical expert come and evaluate the patient and the condition of their organs (Platt & Cascalho, 2013). Once this has been confirmed by the coordinator and death has been declared by the medical staff, the family is then approached, and the topic of organ donation is discussed. Upon obtaining consent from the family of the deceased, the transplant coordinator matches up a recipient based on the location of the donor. Upon choosing an available recipient closest to the hospital, with the most urgency, the coordinator sends the information over to the transplant center to be evaluated. The final decision of whether or not the donor is compatible for the recipient is left to the surgeon doing the transplant. When approval is given from all participants, the process can begin to remove the organs and preserve them until they are able to perform the surgery and transplant them into the receiving patient. The organs are washed and preserved in sterile containers on ice where they are transported to the site of the operation. The length of time each organ can be preserved depends on the organ itself, with some being roughly twenty-four hours and others being only four hours of preservation (Platt & Cascalho, 2013). This process of organ transplantation has

been around for several decades and has proved to be successful with both the preservation of the organs, as well as the procedure of transplanting them into the new patient. While organ transplants give the patient a longer time of survival, they are not a permanent cure for the patient because there is still a foreign organ in their body that will deteriorate over time (Rana & Godfrey, 2019). Several organs, including kidneys, heart and pancreas, have an average lifespan of ten years, while organs such as the liver have an average lifespan of about 5 years (Rana & Godfrey, 2019). There are many different factors that can increase or decrease the length of time that the organ can survive. In the end, the transplanted organ will not last as long as many people would desire, but it increases the projected years of survival for the patient. Overall, organ transplant is a blessing and gift that many are fortunate to receive.

Before the process described above was put in place, organ transplant was not as efficient or effective. With the incredible success rate increasing and organ transplants becoming more common and less perilous than they had been in the past, the U.S. Congress decided to put into place the National Organ Transplant Act in 1984 (Gross, 2008). There was a scarcity of available organs to be transplanted into patients that needed it, and they also had no methodology for finding other patients that needed new organs. For example, if an organ was donated and available for transplant, they only had a small radius of local hospitals and clinics that they were able to use to find a recipient for the organ and if there was no one that qualified then the organ would go to waste. Furthermore, the government sought to develop a system that efficiently and accurately matched the patients with a correct donor (Gross, 2008). This method allowed organs that were donated to be quickly paired with the necessary patient. At the time they were able to keep organs alive and healthy for a time but waiting too long for a transplant resulted in the

inability to use the donated organ for transplants. The National Organ Transplant Act also prohibited and outlawed the sale of organs for monetary value in order to force all available organs to be donated to save lives through the transplant system. This incredible organ act in 1984 allowed the process to be handled at a national level (Gross, 2008). The reason for this stemmed from a desire to be fair and match the newly received organ with the person that needed it the most, eliminating any bias involving all associated parties (Gross, 2008). It removed the risk of having unfair protocols and maintained a professional, reliable and objective standpoint. Over the years, the organ donation system has also improved with technology, specifically with pairing the recipients with the donated organs. It began with using mail-in forms to track the donors and recipients, but with improvements in technology, computers became available. With the computers, they were able to create an electronic database that filed and managed the system better and more efficiently than with paper. With so many patients needing new organs, the organization decided they needed to create another system to place each individual on a list in order of necessity and emergency. This led to the birth of the organ transplant list.

The organ transplant list is a system that was created by an organization in charge of organ transplants in the United States called the United Network of Organ Sharing (UNOS) (Singhal et al., 2009). This network has put together a list of all of the candidates and matches in order to ensure equality and objective matching. The patients at the top of the waiting list are individuals who have been waiting the longest time or are in the most critical need. When a new organ becomes available, each individual on the recipient list is analyzed and compared with the new organ to determine compatibility. They look at components including the genetics, size, blood and tissue type of the organ; how long the patient has been on the waiting list; their need

for a new organ; and the relative distance between the donor and the recipient (Singhal et al., 2009). When the compatible recipients are identified, the patient closest to the location of the donor often gets preference. The process then repeats as each new organ is donated and becomes available for transplant.

Although the organ transplant list has been successful in ranking and pairing recipients and donors, there is still a tremendous shortage of organs available for transplant. The recurring issue is that the list of people needing organs is constantly growing in length, while the number of available organs that are healthy and able to be used for transplants cannot keep up with the excessive need (Satel et al., 2014). Almost 30 years after the introduction of the National Organ Transplant Act, the wait time for organ recipients has increased by roughly five times, beginning at almost a year wait to now 5 years before a new organ that is correctly matched becomes available (Satel et al., 2014). Thousands of individuals have died over the years waiting for a correct and available match. Despite the success stories of patients receiving new organs, there is an almost equal number of individuals that did not survive due to the shortage of available organs. With such a high percentage of mortality correlated to waiting for donated organs, the goal for a long time has been to find a way to lower that number and ultimately eliminate the problem through the use of technology.

The Influence of Technology on Organ Transplant

The innovation of science and research is directly connected with technology. As technology emerges and progresses, so does the ability to advance and increase in other areas such as science and medicine. Over the past decade, there has been an explosion of technology, specifically regarding computers, phones and electronics. However, it has not stopped with

electronics but has also spilled over into the world of medicine. With organ transplants specifically, technology has allowed the transplant list to become more efficient and unbiased due to transferring it to an online database. Even with all of the ways that technology has increased and improved the process of organ transplantation, however, it cannot eliminate the problem of the shortage of donated organs. The transplant system has evolved over the years and been incredibly successful; however, it has not changed the lack of donors (Seetapun & Ross, 2017). Because of this problem, researchers have been searching and developing new techniques that would eliminate the need for transplanting organs.

One of the emerging technologies is known as tissue engineering. Tissue engineering is a relatively new field of study that is a mixture of engineering, science, and medicine (Howard et al., 2008). It is often called regenerative medicine, where stem cells and other living materials are engineered to form new tissue in the body. The entire purpose of engineering new tissue is to use new manufactured cells in place of old tissue that has become damaged, whether from injury or organ failure. In the late 1900s, researchers were able to create the dermis and epidermis of the skin, which was an incredible breakthrough. They had predicted that in the next 15 to 20 years, tissue engineering would have progressed to the point of successfully reconstructing organs; however, they were met by several challenges. The main hurdle they encountered was that they needed to consider the vasculature and correct blood flow to the organs (Berthiaume et al., 2011). The other main problem is that it is incredibly expensive to continue research, so it had been funded by private donors in the past (Berthiaume et al., 2011). However, as research has progressed, small organs have been produced and successfully used in transplants. This is a

breakthrough, specifically with surgeries, because patients needing transplants will not be limited to finding organ donors that match up correctly with their body (Satava, 2008).

Organ Transplants and Tissue Engineering

With the incredible breakthrough of this emerging area of research, a promising future was seen for organ transplants. The reason for tissue engineering being an area of research for transplants is because the future plan is to completely eliminate the transplant list and by doing so, eliminate the problem of not having enough whole organs to be used (Seetapun & Ross, 2017). In order to understand how the transplant list can potentially be eliminated in the future, one must understand the method by which they mean to replace it and how it works. Researchers are looking to eliminate it by the production of new organs from the patient's own cells which will allow for the extension of their health. Medicine has allowed the lifespan of humans to increase with time as new methods are discovered to prolong life. However, with this extension comes other problems that need to be addressed like the need for new organs and the ability for one's body to accept these organs. Even with the advancement in immunosuppressive therapies and medications in place used for organ transplants, they are still not a hundred percent reliable and being on these medications takes a hard toll on the patient's body over time. Doctors, surgeons and researchers realized that they needed to find a way to synthesize full organs from the person's own cells so that the patient would not have the risk of their body rejecting the new organ. There would also no longer be a need to take immunosuppressive medications because the patient would be putting organs and tissues back in their body that had been made from their own cells, so an immune response would not occur (Olson et al., 2011). The concept of tissue engineering revolves around the regeneration of tissues and organs from cells (Olson et al.,

2011). One of the key components is the location from which the cells are taken or the type of tissue transplant.

There are several different sources for organ or tissue transplant (Sakaguchi et al., 2019). These include autograft, allograft, xenograft and alloplast. First, autograft is a tissue graft where the source of the tissue is the person's own personal tissue or skin. Allograft is when the tissue is from another human, whether alive or deceased as with cadavers. A Xenograft is tissue taken from an altogether different species like an animal. Lastly, alloplast is when the tissue itself is synthetically engineered or produced. Tissue engineering falls in the category of the last source as it is synthetically made in the lab. Alloplasts are the most complicated and intricate of the four styles of skin grafts because they are produced without any living element involved (Sakaguchi et al., 2019). An example of an alloplast is the bioactive glasses that are integrated in with the bone that mimics it and allows further growth and reconstruction of the bone. With the foundation of the history and technique of organ transplants layed out, it is now important to get a better understanding of what tissue engineering is and more specifically how it will tie in with transplants.

Tissue Engineering

Tissue engineering stemmed from the idea of alloplasts (Sakaguchi et al., 2019). The purpose is to use materials that connect the biomaterials and cells to heal the body on its own and restore the function of tissues and organs (Sakaguchi et al., 2019). It falls under the category of regenerative medicine, as discussed earlier, where the whole premise is that the body uses outside components to heal itself through the reconstruction of new cells and tissues (Olson et al., 2011).

The body is made up of molecules and cells which make up the tissues, and tissues which make up the organs. Next to atoms, the cells are the building blocks of the entire body. In order to make new tissues and organs, the process must start with the building blocks, or cells (Sakaguchi et al., 2019). The cells group together to create a structure often called the extracellular matrix or referred to as scaffolding. The matrix and scaffolding are key to functions of tissues and organs because they not only provide structure and support, but act as a network in the signaling process (Howard et al., 2008). The signals that the cells are receiving in the matrix control how the cell responds in the body. Researchers and engineers have been able to control how the cells react by telling them to repair the damages from the inside (Howard et al., 2008). Not only have they been able to manipulate the outcome and cause these cells to repair themselves, but they have also been able to create new tissues by using these mechanisms outside the body.

Tissue engineering has many uses including engineering new tissues or organs to replace failing or faulty ones, as well as being used for testing of biocompatibility, specifically with toxicity or pathogenicity (Sakaguchi et al., 2019). Currently, tissue engineering does not fall under the category of patient treatment options. The reason for this is that it is still a new field and there is a significant amount of research, trial, and testing to be done before it can be implemented in human treatment (Satava, 2008). Small organs, such as arteries, bladders, a trachea and even skin or cartilage has been used and implemented in humans. However, these procedures are extremely expensive and at this point in time, still experimental (Satava, 2008). Tissues of more complex organs have been produced in the lab through tissue engineering, but these remain as experiments for further research since they are not functional or ready to be

successfully placed inside a human (Satava, 2008). However, although these structures cannot be fully used for their desired purpose, they are incredibly beneficial to research of drugs and prescriptions. In the past, companies have tested their drugs on 2-D cell systems or cell assays in an attempt to replicate the *in vivo* model of how a human body would respond to the drug (Nam et al., 2015). Even though these experiments have been helpful to understand how the tissues might respond, it is not as accurate as one would hope. The potential complication of this situation is a huge concern because not being able to accurately replicate the environment of the body and cell system *in vivo* means that these companies are not able to precisely predict the response from the body *in vivo* (Nam et al., 2015). It is with some risk that these prescription companies are producing their drugs when the accuracy of their preclinical tests is not adequate (Nam et al., 2015). Studies have been done which demonstrate that 3-D models of these tissues and cultures have a tremendous increase in accuracy when it comes to real life responses to the medications (Nam et al., 2015). These tissue-engineered organs are useful for these cases in research. Not only does it provide more accurate predictions, but because these tissues are synthesized from the individual's own cells, researchers can test medications on the tissues and see how that person's body responds (Nam et al., 2015). This alone is an incredible tool because prior to these engineered models the only way to test a medication or treatment type on a specific patient was to have them undergo the treatment or take the medication and observe the results. With this ability, the process of finding a tailored treatment for an individual is faster, as well as less invasive to their body.

History of Tissue Engineering

The term “tissue engineering” has been used since the implementation of the National Organ Transplant Act back in 1984. However, even before this term was birthed, there were ancient Hindu myths that tell of clones being produced from a person’s blood (Kaul & Ventikos, 2015). Furthermore, in Greek mythology, there is a legend about Prometheus who committed treason and was sentenced to an eternity of his liver being pecked out by an eagle and regenerating itself every time (Kaul & Ventikos, 2015). These are just a few of the examples of myths or tales that contain ideas of a part of a person’s body having the ability to regenerate. In ancient times, regenerative medicine was not established, but there are records indicating that a form of it was used (Kaul & Ventikos, 2015). For example, a recovered Egyptian papyrus contained instructions for regenerating new tissue from wounds by a “scaffold” method. These scaffolds included the use of herbs and organic material, but the idea is still the same. They desired to make a prototypic environment that would cause new cells to proliferate and create new tissue (Kaul & Ventikos, 2015). Regenerative medicine and its associated treatments have developed over the years to produce the methods that are in place today.

During the second half of the nineteenth century, a man named Rudolf Virchow (as cited in Kaul & Ventikos, 2015) stated that tissue regeneration was dependent on cell proliferation, which led to the testing and experimenting of exponentially growing cells *ex vivo*. This proposition was made after another scientist discovered that the regeneration of tissues depended on the types of cells and the environment in which the tissue was surrounded. The studies performed to proliferate cells outside of the body led to the coining of the term “stem cells”, which were discovered through this process (Kaul & Ventikos, 2015). In the beginning of the

twentieth century, roughly 30 years after stem cells were discovered, cell growth was finally achieved *ex vivo* (Kaul & Ventikos, 2015). Alexis Carrel, the scientist who received the Nobel prize in 1912, developed a method for proliferating cells in large quantities. This technique is known as cell culturing, and is used even today (Kaul & Ventikos, 2015). Through his experiments, Carrel also discovered that pieces of an animal's heart, when placed in plasma and embryonic tissue, were able to not only survive, but also continue to grow for an extensive period of time. This was a huge breakthrough and accomplishment for tissue engineering because this supported the hypothesis that a cell is closely dependent on its microenvironment for new tissue growth (Kaul & Ventikos, 2015).

Upon the discovery of the stem cell, scientists quickly began using these types of cells for regenerative medicine due to their ability to adapt and regenerate or repair the old, damaged tissue (Ramsay, 2002). These types of experiments did not progress or gain footing until the late twentieth century. However, once they started, the concept quickly gained momentum with the incredible hope anticipated for the future of medicine (Kaul & Ventikos, 2015). Researchers conducted experiments to find methods through which they could add these growth instructions inside the body for the implanted cells to follow directions leading to new growth. This led to the discovery of the main foundation for tissue engineering through the creation of a biosynthetic matrix, ultimately leading to the design of scaffolds (Kaul & Ventikos, 2015). With this development, researchers now had a firm basis on which to build their experiments and finally produce tissue and, eventually, organs.

One of the first successful attempts involving tissue engineering was by a man named Howard Green (Nam et al., 2015). He was able to reconstruct the epidermis from his patients'

biopsies by extracting the cells of the epidermis, specifically keratinocytes, and causing them to multiply rapidly (Nam et al., 2015). This was the first success of many that increased the momentum and excitement for tissue engineering. Eventually, tissues were being produced through the synthesis of scaffolds and extracellular matrix prototypes that were able to replicate the tissue when implanted and combined with the cells (Vacanti, 2006). The very first human implant from engineered tissue was in 1991 (Vacanti, 2006). The engineered implant contained a scaffold that was synthetically produced and seeded with chondrocytes. The patient had Poland Syndrome where his chest was not developed, and he lacked a sternum. The purpose was to see if the engineered scaffolding would cause new growth to occur inside the patient's body (Vacanti, 2006). The procedure was successful, and the synthesized scaffolding integrated into the patient's body and the results were better than expected. As time progressed, similar procedures occurred across the world which supported the idea that this new regenerative medicine could be successfully implemented for the replacement of human parts (Vacanti, 2006). Over the years many technological advances occurred leading to the production of whole organs, which is the basis and purpose for tissue engineering.

Components of Tissue Engineering

With a basic understanding and knowledge of the background of tissue engineering, it is important to define the parts that allow tissue engineering to exist in further depth. To begin, there are roughly three components that allow tissue engineering to exist and succeed: the scaffolding, the cells and the molecules. Without any one of these elements, the process of tissue engineering cannot work.

Scaffolds

The scaffolds act as a template or environment for the regeneration of the tissue (O'Brien, 2011). These constructions are incredibly important for the proliferation of the cells. They are intricately composed of fabricated biomaterials with key requirements that must be followed when synthesizing them (O'Brien, 2011). The first factor is that they must be biocompatible. This refers to the scaffold's ability to proliferate the cells and cause them to adhere to the structure, even prior to being placed inside the body. It also must not induce an immune response that leads to inflammation. If this occurs, then the healing process will be prolonged and will often lead to a rejection of the scaffolding and new material by the body.

Secondly, the material that the scaffold is composed of must be biodegrable, so that once placed inside the body it will provide structure but ultimately be broken down. The entire purpose of tissue engineering is to cause an individual's cells to regenerate their own extracellular matrix to heal their body with the cells that are already inside them. However, they need a blueprint in order to start the process of engineering the matrix, which is where the scaffolding comes in play. Once implanted inside the body, the cells will continue following the instructions of the scaffold but over time produce the matrix on their own. Since the scaffolding is not needed long term, it is important to compose it of materials that are able to be degraded and removed from the body without any problems or negative effects.

Another key factor is that the scaffolding must be porous to allow the diffusion of elements and nutrients throughout the structure, as well as allow the cells to penetrate it for further proliferation. A difficulty that has been observed with this component is that the pores of the scaffold must be big enough to allow the cells to enter the structure in order to bind with the

ligands inside. Simultaneously, they must maintain a large enough surface area for cells to bind on the outside in greater numbers. This is critical for the integration of the cells with the scaffold, because this is the method by which the cells cause regeneration of the tissue.

Lastly, the most important requirement to consider when constructing the scaffolds is the material with which to develop them. There are three categories of biomaterial used for constructing scaffolds: synthetic polymers, ceramics and natural polymers (O'Brien, 2011). Each of these groups have their own advantages and disadvantages. The choice of which biomaterial to use often depends on the type of tissue that is being regenerated inside the body. For example, ceramic scaffolding is frequently seen in the engineering of new bone growth, because the material is characterized by its low elasticity, brittle surface and stiffness. These features are ideal for bone regeneration due to their similarity in structure. However, there are downsides to using this material. Many of the issues stem from the brittleness and the inability to mold or shape the material and the newly engineered tissue.

The most promising biomaterials are the natural polymers due to their ability to promote growth, adhesion and proliferation of the cells. The natural polymers also have the best response for degradation because there are no fatal byproducts produced with their breakdown. This allows the cells to create their own extracellular matrix with time due to the ability to degrade the destructive residual material. The issue presented with natural polymers is that their mechanical properties limit their ability to be used as biomaterial for certain tissues that need to have a weight-bearing ability, specifically with application to orthopedics (O'Brien, 2011). Furthermore, the vasculature of the scaffolds is a major challenge and is one of the areas in need of further research. There is still substantial investigation that needs to be done to produce the

most efficient, functional scaffold. However, there has been major success with the methodology of these structures and even an ability to integrate them into clinical trials.

There are two main approaches in which researchers are engineering tissue (Howard et al., 2008). The first is where the scaffolding can be used as a cell support system. The cells are seeded outside of their normal conditions and directed to begin the process of creating the materials needed for tissues. These genetically engineered cells are the starting material for the rest of the tissue. The second approach is where the scaffolding is used as a growth factor for the implanted cells so that new cells can be drawn to the site and create new growth. The cells that are most successful in tissue engineering are primary cells which are taken directly from the patient. Researchers hope that they will be able to combine growth factors with stem cells to allow the tissue and cells to regenerate and cause new growth. Researchers have also tested and found that biomaterial scaffolding is more than just structural support in constructing tissues. It is a way to integrate new modified tissue and cells into the body where new growth needs to occur. They have tested and are continuing to test the different methods of using biomaterial to reconstruct cells. Researchers are beginning to genetically manufacture new tissue with the extracellular matrix by creating biopolymers, proteins, and smart polymers. With the next stage of tissue engineering, researchers are hoping to discover more bioactive materials that will be useful for future endeavors (Furth et al., 2007).

An emerging approach for tissue engineering that has had an increase in its development relates to the reconstruction of damaged osteochondral interface, or the cartilage-bone interface. The osteochondral interface is a difficult area for engineering new tissue because the tissue of both the cartilage and the bone are very different and need separate, specific composition.

However, several types of scaffolding have been developed that will hopefully aid in growth and regeneration for both sides of attachment. There are four proposed scaffolds being tested by researchers within the osteochondral interface to see which option has the greatest success rate. The make-up of the scaffold is incredibly important because there are many qualities it must possess. One of the main limitations when developing cartilage tissue is that it has a low self-regenerating power. For osteochondral regenerative tissue, it must have actively proliferating cells, and so many have suggested and incorporated the use of mesenchymal stem cells (Nooeaid et al., 2012).

Cells

The next key components in the process of tissue engineering relates to the cells that form extracellular matrix. Prior to researchers developing the idea of engineering whole organs, guided tissue regeneration existed (Sakaguchi et al., 2019). The procedure consists of an injection of cells from the patient into the site of injury. The process causes the desired cells to proliferate in the area, which creates a barrier preventing the unwanted cells from growing and developing. Once scaffolds were introduced, the methodology for engineering tissues changed. The cells now had an easier process for proliferating and producing an extracellular matrix for new tissue growth because scaffolds provide a surface area and contain a porous membrane. These components allow the integration and adhesion of the cells leading to their rapid increase in numbers. Initially, the scaffolds are seeded *in vitro,* where cells are spread on the structure and encouraged to adhere and grow. This process is performed on a media with nutrients and growth factors which are necessary for the growth and establishment of the tissue (Sakaguchi et al., 2019).

The cells being used for tissue engineering come from the patient or individual in need of new tissue. The reason that the cells are collected from the individual is because when the new tissue is engineered and able to be integrated back into their body, there is a significantly lower risk of producing an immune response. As discussed earlier with organ transplants, the patient must first receive immunosuppressant medications in order to repress their immune system so it will not reject the new organ being integrated into their body. The beauty of tissue engineering is that since the person's own cells are producing the matrix and ultimately the tissues, the body should not reject its own cells. The cells being used for this technique are stem cells from the patient, often adult stem or hematopoietic cells (Olson et al., 2011). These cells can be extracted from the bone marrow, adipose, skeletal muscle and gastrointestinal tract. Other stem cells that have been looked at for research include embryonic stem cells (Ramsay, 2002). They are found in a blastocyst, in the inner mass of the cell, specifically in humans. However, there is much debate in the science realm regarding the ethicality of this matter because upon extraction of the embryonic stem cells, the blastocyst is eradicated.

Cell culturing is important for tissue engineering because the cells must expand in numbers and grow exponentially in a lab prior to integration in the body. Normal cell culturing is different compared to culturing with 3-D scaffolds (Sakaguchi et al., 2019). It is important for the seeding of the scaffold to be done quickly and efficiently, ensuring that all areas of the scaffold are equally distributed with the cells because a uniform tissue is the desired outcome. The integration of the cells to the scaffold in the lab is a key component in the process of seeding because it ensures the reproduction of functional tissue that will continue to develop once inside

the body. Along with seeding, it is important to use growth factors that result in even, exponential growth across the structure.

Stem Cells. The discovery of stem cells was an incredible accomplishment for the world of medicine. They have become one of the leading reasons for the development of tissue engineering and regenerative medicine (Gomillion & Burg, 2006). Stem cells have revolutionized regenerative medicine because they are cells that they have not differentiated into a specific type or subset of cells in the body (Bianco & Robey, 2001). They also have incredible ability to expand and proliferate rapidly. The fact that these cells are not specialized makes them incredibly beneficial for use in tissue engineering. They have the ability to self-renew and can be used in any area of the body, specifically in sites needing repair. Stem cells, after migration to the sites of need, are able to produce progeny cells that are capable of forming different tissue types in the body (Sakaguchi et al., 2019). With the appropriate signals, stem cells can differentiate into mature cells with specializations for specific regions and tissues. These cells also possess the ability to propagate in a lab, which is important for proliferation *in vitro* with the scaffolds. There are several different types of stem cells but the one that has proven most efficient and productive are the mesenchymal stem cells.

Mesenchymal stem cells are superior materials to use in tissue engineering. They are found in the bone marrow of adults and exist as one of the subsets of multipotent stem cells. Multipotent are the more restrictive stem cells as they can only produce daughter cells that are specific to a couple types of tissues (Rosenbaum et al., 2008). However, these specific stem cells are used frequently due to their ease of isolation (Rosenbaum et al., 2008). They are complex cells that can split into different mesenchymal types, such as ones that can be used to make

specific bone marrow tissue. These cells have greatly helped tissue engineers because they are capable of being used in cartilage, bone, tendons, and ligaments, as well as other connective tissue. Furthermore, mesenchymal cells are constantly being produced and proliferating while others are simultaneously dying due to their lifespan. This is an important aspect because the cells that make up tissues progress rapidly with time and need new cells to be produced. For new tissue engineering, researchers are creating a modified cell and replacing these fresh, new cells with the dying ones during the turnover cycle. They have used the mesenchymal cells and transferred them to locations that need repairs such as injuries or other tissue-damaged sites (Caplan, 2007). While these stem cells are not as versatile as embryonic stem cells, researchers have preferred to use them because they are successful with tissue engineering and they are not having to face a moral and ethical dilemma.

Growth Factors

The last key component to the success of tissue engineering is the use of growth factors and cytokines. Growth factors are specific proteins that promote growth and proliferation of tissues or cells (Chen et al., 2010). Furthermore, they have the ability to send signals and maintain communication between cells and the extracellular matrix. They are important in the process of cell differentiation and tissue repair, which is why they are necessary for the engineering of tissues. Pertaining to wound healing, growth factors direct and act as instructions for the regrowth of new tissue through repair. They work through pathways and trigger the signaling of many of them. Because growth factors are important to signaling pathways, the number of receptors dictates how responsive the cell is, as well as how it responds (Chen et al., 2010). Cell proliferation is based on numerous factors, most of them involving the environment

and nutrients. However, growth factors are among the key ingredients needed for proper expansion. The goal for tissue regeneration is to mimic the environment of the body specifically with the development of tissues and cells (Chen et al., 2010). Thus, it is important for the integration of growth factors to replicate that ideal, imitated environment. One of the ways in which tissue engineering has approached reproduction is by creating a replica of the cascade of wound healing (Chen et al., 2010). This cascade cannot occur without the help of growth factors to ensure proper development of new healthy tissue to replace the old, damaged parts. They stimulate the body to repair the site of the injury, signaling for the correct cytokines and cells to migrate to the location and perform their functions. In order for the growth factors to function properly, they must be able to travel to the site of injury prior to degradation and upon reaching the location, live long enough to complete their course of action (Chen et al., 2010). This functions properly inside the body, but when translocated to laboratory settings, the approach is a bit different. Researchers have concluded that they must enable other signaling molecules to help initiate the signaling cascade for wound healing in order to properly ensure the correct responses (Chen et al., 2010). The techniques for delivery of the growth factors to the cells and scaffolding is an area that needs further research to discover the best option. The key growth factor families that have been found to effectively aid and function properly with tissue engineering have been the transforming growth factor – beta, hepatocyte growth factor, connective tissue growth factor, and several more families.

Final Process of Tissue Engineering

The process of engineering the actual tissue is an intense process. To begin, the scaffolding must first be developed (Godbey & Atala, 2002). The detailed production starts with the choosing of the biomaterial to use as the basis of the structure. Once the scaffold is put together, the cells must then be extracted, whether from the patient or another source. The cells are then seeded onto the scaffold, and the entire 3-D model is incubated in specific mediums that contain nutrients and growth factors. This process allows the cells to adhere to the scaffolding and proliferate across the surface, creating the extracellular matrix, which forms the tissue. After this has occurred outside the body, the entire model can then be integrated into the body at the desired location. The scaffold will eventually be degraded down and eliminated from the body, leaving the cells to continue to regenerate the new tissue. Researchers have been able to produce full organs through the same process as previously described.

Conclusion

Tissue engineering is an emerging field of treatment in medicine. It provides many patients with wounds, injuries, and scar tissue to heal faster and more efficiently. It is a type of regenerative medicine that is promising in many aspects, including future treatments like surgeries and transplants. There has been an incredible amount of success that has sprung from this form of medicine; however, there is still significant research and testing that must transpire before it gets to the desired goal. The future for tissue engineering is very promising as it will eventually remove the need for a transplant list, which will be an amazing feat for the world of medicine and mankind. There are still many questions needing to be answered such as long-term

effects and potential problems with this technique, but advancements in technology and research will provide the basis for the future of tissue engineering.

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