BIOLOGICAL ENGINEERING: ADVANCES AND METHODS

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ABSTRACT

The last few decades have seen a tremendous growth in the field of bioengineering. As the need for further treatment and innovation for tissue repair, partial to full organ replication, and gene therapy continues to increase, the field of bioengineering will be tasked with curing and preventing disease and traumatic injuries. The two primary fields currently being focused on in the lab are the way cells interact and communicate to build tissues, and the nature and materials utilized in scaffolding to allow differentiation and migration when cells are seeded. Within those two fields are subsets of different methods, materials that vary greatly. Some stem cells offer certain benefits, yet lack viability due to a host of obstacles, such as ethical questions about their procurement, to their technical obstacles, such as materials utilized for best profusion in a scaffold. It appears that proper and adequate funding for research into finding solutions will be pivotal in having the next medical breakthrough in science. It may very well be referred as one of the greatest advancements in modern history and forever change the face of science should this technology become successful and accessible. Indeed, recent successes in patients would be a strong indicator that this technology and innovation is not too distant in the future.

INTRODUCTION

Currently, in the US alone, there are over 100,000 candidates awaiting organs on the national organ waiting list. On average, a person on that waiting list dies every 90 minutes (UNOS 2012). In addition, as the amount of recipients is increasing, the amount of donors is decreasing. This is due to factors such as ethical debates and customs regarding organ donation, and the fact that organs may only be harvested coincident with brainstem death, which necessitates hospitals utilize additional resources to keep patients on life support (Briggs et al.1997). While technology and advanced methods have fine-tuned the science of organ transplants, it does not address the need for more organs. In addition, tissues such as cartilage, muscle, and even neural tissue for regeneration in vivo can have a tremendous impact on overall life quality in a patient. In response to this need, the last 30 years have been a whirlwind of activity for researchers to try and replicate and build new tissues and organs, thus creating the field of biological engineering. An early example of tissue engineering was developed by Dr. J. Burke of Massachusetts General Hospital. He created a synthetic neodermal skin utilizing chrondroitin 6-sulface in tandem with collagen, to cover burn patients while their skin was in the process of regenerating.

Bioengineering is multifaceted and has many working and intrinsic parts. It requires an in depth and interdisciplinary understanding of the biological cell such as its embryonic origin, cycle, replication, metabolism, energy requirements and proliferation stages, as well as a keen understanding of structural engineering, materials unique in biological adsorption or absorption, chemical compounds and nanotechnology to pair them together in a hybrid organ.

At the fundamental level, it begins with the cell, specifically using an embryonic stem cell or mature adult progenitor cell (MAPC) to direct and grow. However, having cells proliferate is not enough, as a clump of differentiated cells is all that would be present, hence the need for scaffolding as well. Scaffolding is tasked with providing a three dimensional structure for the cells to grow on and to deliver growth factors to nurture the cells. Study and research analyzing embryonic and adult stem cells and its methods of use, as well as the requirements scaffolding need to become viable, and assess and evaluate which of the many scaffolding

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materials/methods currently being tested, hold the most promise for full organ replication in the near future.

1. Stem Cell Role in Regenerative Engineering

Stem cells were discovered to have the ability to replicate and divide into any type of cell in the body (plasticity). This opened up the possibility for regenerating diseased tissue or failing organs by reseeding those areas with stem cells to differentiate and proliferate to become the new cells in those areas. When combined with the proper method of feeder lines and guided cell growth, the development of the cell can be accomplished. There are several different types of stem cells, each with its own unique set of benefits and characteristics. Some exhibit obstacles as well, and are in the process of being studied.

Human Embryonic Stem Cells

Stem cells isolated from the embryo are achieved in chronological proximity to egg fertilization. These cells are derived from the inner mass of the blastocyst cell after four to six days of gestation, at which point they number approximately one hundred fifty cells, comprised of none differentiated and totipotent cells. They will continue on to form the more than two hundred tissue types in the human body. Derivation is executed through several means. The primary and most successful method utilized, is by removing the outer trophectoderm via immunosurgery thus exposing the inner mass cell (ICM) for disaggregation and plating on a feeder cell layer for further culture. Cultivation of stem cells is maintained by providing the proper nutrients such as proteins and growth factors, which can be efficiently done if cells are in a suspension medium, specific to the cell's needs. At day six, these cells will enter the blastocyst stage and have differentiated into trophectoderm and an inner cell mass that includes the progenitors of all the cells in the body. Thus the embryonic stem cell has tremendous potential for differentiation into any type of cell needed, and is unique in its capacity to propagate without losing pluripotency, thus may readily differentiate into various cell types of the three embryonic germ layers. An additional advantage of human embryonic stem cells is the relative ease with which they can be genetically engineered by a broad range of techniques such as transfection, electroporation, and viral infection (Power and Rasko 2011). Human embryonic stem cells have the additional advantage of rapid mitosis as well, and can divide at an exponential rate. However, unless the stem cell is banked from the recipients own stem cells, rejection is still possible and quite likely. Additionally, there are many ethical debates about whether utilizing embryonic stem cells. Primarily, the concern seems to be that according to one school of thought, life begins at inception, so extracting cells from a fertilized egg is akin to destroying life (Briggs et al. 1997). Additionally, due to its extreme proliferative nature, they often can become cancerous and form teratomas. Indeed, there have been documented cases of tumors containing fully formed teeth or retinas.

On the other hand, there are many advantages to utilizing embryonic stem cells. Primarily, these cells exhibit the ability to unlimited or prolonged self-renewal and the potential to produce all the differentiated cell types necessary for any specific tissue. Their embryonic state allows for exponential growth that can propagate, assuring robust growth. Due to their ability to differentiate into any type of cell, all tissue types can be generated. Additionally, the extreme plasticity of the cell makes it an ideal candidate for programming the cell DNA to express the specific differentiation desired. However, although human embryonic stem cells are not yet differentiated, they are already coded in their DNA for the proteins and markers they will exhibit. This can lead to host rejection should an immune response be initiated. Creating a cell bank with all the different types of HLA (human leukocyte antigen) to act as a reserve for

matching the specific needs of recipients would certainly address the issue of host rejection and would drastically decrease the amount of time a recipient would need to wait for the quorum of cells needed for transplant (Cabrera et al. 2006).

MATURE ADULT PROGENITOR CELLS

Mature progenitor cells have proven most successful in the clinical aspect. This is due to its already partially differentiated state, which makes the manipulation and direction of differentiation more viable. Mature adult progenitor cells do not illicit host rejection, when it is the recipient's very own cell. However, it does have certain limitations. The main obstacle with progenitor cells, is that they are already differentiated, thus making them only viable for their specific cell of origin. Additionally, sometimes the area where the progenitor cells may be from, are in a necrotic state and viable cells for extraction is no longer possible. When that is the case, receiving progenitor cells from a donor will result in the same problems of host rejection due to different phenotype. (Shokier et al. 2010). Additionally, the use of stem cells eliminates the need for lifelong immunosuppressant drugs as the stem cells utilized for regeneration is the recipient's own cells. Additionally it has been found that when these cells are encapsulated with Fibrin, they secrete more collagen. (Lin et al. 2010)

Mature adult progenitor cells are primarily selected from bone marrow derived mesenchymal stem cells (BMSCS) which are capable of differentiating into a number of different cell types of mesoderm lineages, including adipocytes, osteocytes, chondrocytes, and other mesodermal cells (Fillmore et al. 2005). They are generally cultured by using colony forming unit fibroblasts. Raw or ficoll-purified bone marrow is placed onto the plates. Mesenchymal stem cells can exhibit signs of adherence to the tissue culture plastic within 1-2 days. Hematopoietic cells do not adhere. Also, flow cytometers are used to filter for STRO-1 markers. Lin and Bo experimented with mature adult progenitor cells to see how well they differentiate into hepatocyte like cells. They used a 21 gauge needle inserted into the bone which was then flushed with Dulbecco's modified Eagles medium (DMEM). The cells were then centrifuged with a Ficoll step gradient of 1.077g/ml at 1500 rpm. Mononuclear cells were then collected and re-suspended in Dulbecco's modified Eagles medium with 10% fetal bovine serum (Fillmore et al. 2005). This process was done for both chondrocytes and osteoblasts. For chondrocytes, enough extracellular matrix was formed to adequately plug areas of tissue damage in knee joints. For osteoblasts, mineral deposits were observed to show that the osteoblasts were indeed calcifying the extracellular matrix and creating bone. This has had strong clinical success and will hopefully be available as bone replacement in the near future. It is also important to note that mature adult progenitor cells are currently the only type of stem cell approved by the FDA for medical application. This is mostly due to the fact that as mature adult progenitor cells or only multipotent, they do not exhibit the exponential growth of embryonic or cord derived stem cells, thus not posing a risk of cancer or teratoma formation. However, the main obstacle of mature adult progenitor cells are they are very limited in clinical applications due to their very stable properties. It has been speculated that with dedifferentiation, this obstacle might be overcome. Through several unique pathways, namely the NOGGIN pathway, it is possible to reprogram an already differentiated adult cell back to its basic non-differentiated state, thus having the same capabilities as embryonic stem cells, but not have the rapid mitotic characteristic that can become cancerous. (Cai et al. 2007). Dedifferentiation is not a farfetched concept as it is seen in many amphibians, animals and even humans. Amphibians, have exhibited the ability to regenerate damaged organs to fully functional states (Davenport 2005). This makes

a strong argument for dedifferentiation to be funded and researched extensively, as it might hold the key for repairing damaged organs in humans as well.

CORD-DERIVED MESENCHYMAL STEM CELLS

Data supports the indication that cord derived mesenchymal stem cells are a unique combination of the positive qualities of both adult progenitor and human embryonic stem cells, with none of the disadvantages either of those cells exhibit. They are extremely proliferative due to its embryonic nature (not quite as proliferative as human embryonic stem cells), yet have developed enough to exhibit signs of mature progenitor cells. This allows them to be programmed to differentiate into specific types of cells. Additionally, due to their chronological proximity to their phenotypic state, it is quite possible that certain cells are original stem cells much like those found in the human embryo. Drawbacks include possible rejection by host because they already have markers. Much like the idea of a human leukocyte antigen bank for human embryonic stem cells, the same concept would benefit mesenchymal cells, allowing for quick and accurate matches specific to the host. Due to the combined benefits of cord-derived cells, it would seem that extensive funding and research would yield many advances and discoveries in gene therapy as well as the field of regenerative medicine. It is also important to note that the nature in which these cells are derived pose no ethical quandaries, as these stem cells are derived from discarded placenta. Additionally, they are relatively easy to isolate and can be utilized in the lab at minimal cost.

2. SCAFFOLDING

While cells may proliferate and grow, they need to be guided and assembled in a proper dimensional order. This can only be achieved with the proper scaffold to direct it. Primarily, to meet the specific needs of the cell, the scaffold must be porous, adhesive, biodegradable, and include a mechanism for nutrient supply.

Porosity insures that nutrients can get to the cell and waste can be removed. Adhesion will allow for the cells to adhere to the scaffold so that it may grow in the direction desired. Additionally, in vivo, this prevents encapsulation of implanted tissue, thus allowing nutrients and wastes to be exchanged. Biodegradability will insure that the scaffold will disappear so that remnants do not become toxic and mechanical functions perform properly. Finally, the scaffold should be made of materials that function to supply nutrients to growing cell so that all cells that adhere are being fed.

It is crucial to note, however, that simply seeding the cells on a scaffold will not suffice. Cells operate and communicate with each other from early on, and even though cells may be of the same lineage, they have adapted different roles in any given system. For example, hepatocytes have different functions based on their location in relation to their oxygen gradient. Some tasked with being glycolytic hepatocytes can be transformed into gluconeogenic hepatocytes. Therefore, in a lab environment where oxygen perfusion is at a 100% homogeneously, it would result in cells not functioning to their specific characteristics (Lenas et al. 2008). A thorough understanding and study of cell mimetics is important in order for scaffolding to be effective.

BIOLOGICAL SCAFFOLDING

Through the process of decellularization, cells are stripped of the extra cellular matrix leaving behind just fibers and collagen. When applied to fully formed tissue or complete organs, the only left behind is a translucent, or ghost, extracellular matrix. This is ideal, as within the extracellular matrix all the intricate vascular and collecting networks are left in place. This allows for the reseeding of those areas to be more specific and accurate. Additionally, this

process serves to leave all mechanical functions working properly, and retains all the natural mechanics of the organ. In fact, in a study published by Nature Medicine, after eight days of reseeding a decellularized rat heart, it was able to conduct complete electrical myocardial impulses resulting in complete ventricular contraction as was demonstrated on an EKG (See Figure 1).

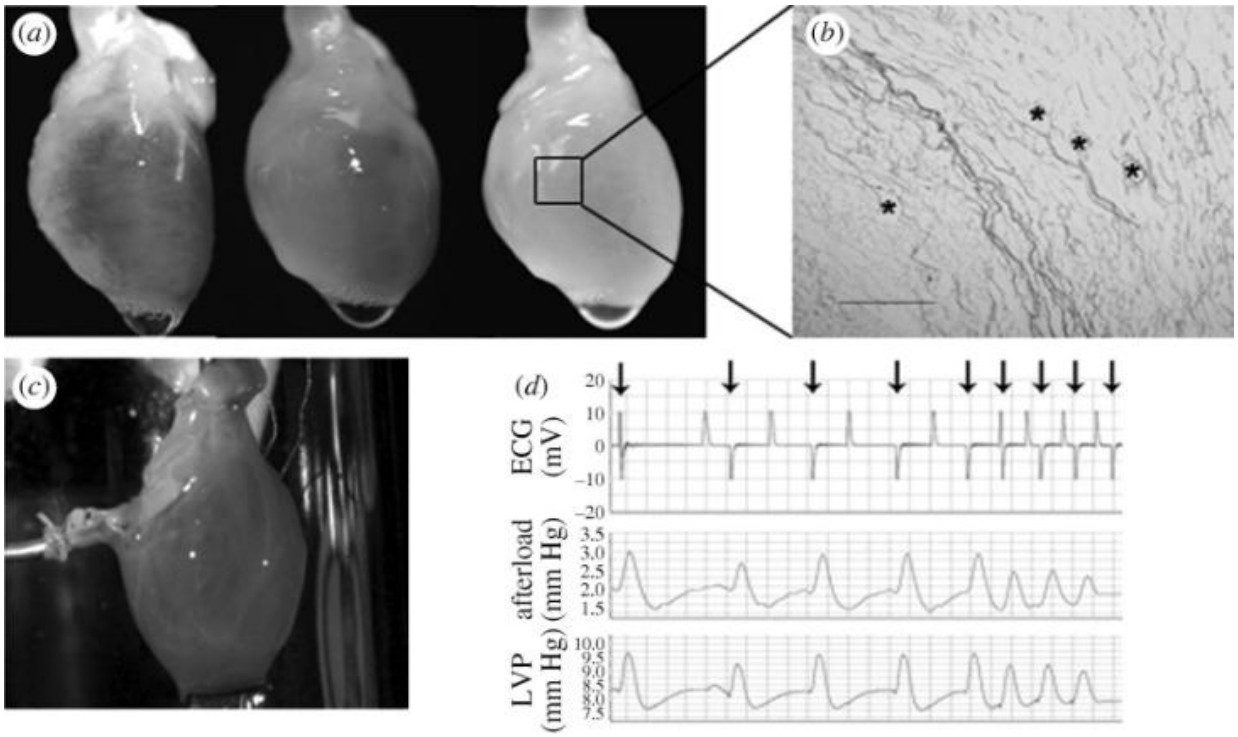

Figure 1: Photographs of cadaveric perfusion decellularization of whole rat hearts perfused with sodium dodecyl sulphate (SDS) over 12 h. (a) The heart becomes more translucent as cellular material is washed out from the right ventricle, then the atria and finally the left ventricle. (b) Haematoxylin–eosin staining of thin sections of SDS-treated heart showing no intact cells or nuclei. Maintenance of large vasculature conduits (asterisks). Scale bar, 200 µm. (c) Formation of a working perfused bioartificial heart-like construct by recellularization of decellularized cardiac extracellular matrix. Recellularized whole rat heart at day 4 of perfusion culture in a working heart bioreactor. (d) Representative functional assessment tracing of decellularized whole heart construct paced in a working heart bioreactor. Tracings of electrocardiogram (ECG), aortic pressure (afterload) and left ventricular pressure (LVP) of the paced construct on day 8 after recellularization, and on day 8 after stimulation with physiological (B50–100 mM) doses of phenylephrine. Source: Ott et al. 2008

The cells are stripped of the extracellular matrix through the utilization of detergents – most commonly sodium dodecyl sulphate. However, it has been discovered that sodium dodecyl sulphate may destroy up to 80 % of the collagen found in the matrix (Petersen et al. 2012). To counteract the chemical destruction of part of the extracellular matrix, TritonX-100/sodium-

deoxycholate was added. In a study done by Drexel University, researchers were able to combine sodium-deoxycholate with sodium dodecyl sulphate to achieve minimal to no extracellular matrix loss (Fitzpatrick et al. 2010). Additionally it was discovered through the use of electroporation, cells were able to be lysed without damage to the extracellular matrix. Electroporation is defined as a "non-linear biophysical process in which the application of pulsed electric fields lead to an increase in permeability of cells, presumably through the creation of nanoscale pores in the lipid bi-layer. At low pulsing energy, this permeability is reversible and cellular health and function is maintained. Once a critical electric field intensity threshold is surpassed (approximately 500 to 700 V/cm for ninety 50 μs pulses at 4 Hz in the brain and eight 100 μs pulses at 1 Hz in the liver, respectively),the cell membrane is unable to recover and cell death is induced in a precise and controllable manner with sub-millimeter resolution" (Sano et al. 2010). This process is referred to as non-thermal irreversible electroporation (N-TIRE). Clinical successes include the first woman to receive a tracheal transplantation seeded with her somatic mature adult progenitor stem cells in 2011.

However, the main drawback to biological scaffolding is the limited supply of donor organs to strip cells of. Even more importantly, specific dimensions needed for implantation is not possible with already formed biological scaffolding. In the case of a damaged right ear that needs to be matched with the remaining left one, exact dimensions and proportions are required. Clearly this would not be possible with scaffolding that is already shaped. It should be noted though that decellularization has had the most success with tissues that do not require specific dimensions, like a trachea.

SYNTHETIC SCAFFOLDING

Synthetic scaffolds can be comprised of either biological (natural) polymers such as chitosan, collagen, and polycaprolactone (PCL), or synthetic polymers such as poly(α-hydroxyacid) and polyglycolic acid (PGA). Most utilize some form of polylactic acid (PLA) and their copolymers (PLGA). There are a variety of techniques that have been utilized and reviewed for processing three-dimensional porous scaffolds. (Ikada 2006). Although it has been shown that synthetic polymers have advantages over natural polymers, such as straightforward control of bioabsorption rate and tunable mechanical properties, their surfaces are hydrophobic, thus preventing cell and tissue adhesion and requiring proper treatment to insure that these synthetic scaffolds absorb culture medium (Tamada et al. 1986). Additionally, the rate of degradation can be controlled and timed to coincide with cell to cell adherence when scaffolding support is no longer necessary (Jovanovic et al.). This process of hydrolytic degradation can be obtained via two methods. One is done by bulk degradation, and the second method used is through the process of surface erosion. "Bulk degradation can be observed if during random chain scission, an overall decrease of molar mass is exhibited. Surface erosion, is the process where hydrolysis removes only polymeric chains from the outer layer of the material thus leaving the bulk of the material untouched" (Yang et al. 2007). Surface erosion is favored for many applications of polymeric biomaterials due to the material properties remaining virtually intact, as degradation proceeds through removal of very thin layers of the material. This insures that the scaffold is no longer present to hinder any possible affects on mechanical function or toxic interference with cell communication. Most importantly, synthetic scaffolding offers the obvious advantage of precise and custom dimensional properties. This precision clearly is a need for partial tissue or organ repair that requires specific sections to be replicated. Additionally, exact proportions might be utilized for aesthetic reasons as well, such as seeding a scaffold with cartilaginous tissue in order to build one ear so that it complements the other ear. Furthermore, availability is a non

issue as supply is not dependant on any other factors other than procurement of the materials needed. The biggest obstacle that needs to be overcome is functionality. The scaffold designed must meet all the requirements outlined in order to allow the cells to proliferate and migrate in the proper direction. Of the many scaffolding that has been experimented with, only a select few have had much success in the clinical phase. With many materials to experiment with, the process is mostly done by trial and error with eventual successful results. It is with this fact that it seems the next big breakthrough is bound to happen relatively soon.

CONCLUSION

There is no doubt that the science of biomedical engineering still has much to accomplish in making the technology a widely accessible application in the clinical setting. Though cord, or placental, stem cells seem to offer the most promise for continued groundbreaking in the regenerative field because they lack many of the obstacles faced by embryonic and adult stem cells, currently, the use of mature adult progenitor cells is the only one approved by the FDA for cell therapy. A beneficial step forward for stem cells would be to create a human leukocyte antigen bank that has all cell type/markers available so that regenerative growth can be done quickly with no need for immuno-suppressive drugs due to human leukocyte antigen matching. Cells, when combined with synthetic polymer scaffolding, can proliferate and take up their three-dimensional shape freely. Indeed, the proper treatments of the scaffold must be maintained, and biodegradation via lysozymal activities and pathways must be closely monitored to insure proper profusion with minimal toxicity. With clinical trials currently underway, the near eventuality of full organ replication looks promising.

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