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The Future of Cell Therapy and Tissue Engineering in Cardiovascular Disease: The New Era of Biological Therapeutics

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1. Introduction

Cardiovascular disease remains a major cause of morbidity and mortality worldwide, resulting 16.7 million deaths each year, accounting for 29% of all deaths globally (WHO, 2006). The major modality of death due to cardiovascular disease remains acute myocardial infarctions (MI) and sudden cardiac death with more than one third of first time MI not making it to the hospital. MIs are mostly caused by sudden occlusion of a coronary artery secondary to plaque rupture and acute thrombosis resulting in loss of oxygen supply to the territory affected; if flow is not restored in a timely fashion, myocardial death ensues. The resultant of that is loss of contractile function, cardiac remodeling and eventual heat failure. The resultant compensatory mechanism however, is cardiac remodeling with fibrous tissue replacing the damaged myocardium early on and overcompensation of the remaining myocardium to maintain cardiac output. This eventually leads to negative remodeling, ventricular dilatation, and eventual loss of pump function and heart failure ensues. Furthermore, fibrous tissue deposition and structural remodeling of the heart in the setting of heart failure leads to electrical uncoupling of myocardium from fibrous tissue and development of re-entrant electrical circuits that lead to the development of significant ventricular dysrrythmias. This increases the risk of sudden cardiac death in patients with heart failure whether this is due to myocyte loss from heart attacks or other causes of heart failure (Leor J 2000; Anversa P 2002; Li SC 2009). Scar formation secondary to cardiac remodeling and fibrosis results in increased risk of fatal dysrrythmias as this tissue becomes a nidus for dyrrthmia origination and propagation. (Strauer BE 2002; Stamm C 2003; Galiñanes M 2004; Pittenger MF 2004; Wollert KC 2004; Dib N 2005; Dimmeler S 2005; Patel AN 2005; Deindl E 2006; Engelmann MG 2006; Schächinger V 2006; Tse HF 2006; Wu KH 2006; Kissel CK 2007; Stamm C 2007; Theiss HD 2007; Yerebakan C 2008).

Although there have been great advances in the knowledge of the mechanisms and treatments of myocardial, therapies for cardiac regeneration and repair are still not available for end stage heart disease. Our current treatments for heart failure are only temporizing

whether it is medications such as beta blockers, angiotensin converting enzyme (ACE) inhibitors, diuretics and other classes or devices such as implantable defibrillators and bi-ventricular pacemakers (Moss AJ 2009). Once end stage heart failure ensues, one year survival rates can be as low as 50% for New York Heart Association (NYHA) class III and IV heart failure; the only options then to restore pump function are mechanical ventricular devices (VADs) (Birks EJ 2006) as destination therapy or ultimately open heart transplantation. However, due to the high cost of VADs and the shortage of donor organs these are not viable options as demand for a transplant is ever increasing with a relatively fixed supply of donor hearts.

The concept of the heart as being a post mitotic organ with no cardiomyocyte cell generation and turnover has been challenged over the past decade with culminating evidence that there is slow process of cell turnover in the heart postnatally. This process appears to be slow with a 1% per year turnover rate at the age of 20 decreasing to about 0.45% per year at the age of 75. Fewer than 50% of cardiomyocytes are exchanged during a normal life span (Bergmann O 2009). Although there appears to be postnatal cardiomyocyte turnover and generation, this is insufficient to replenish lost heart cells (myocytes) post MIs and in the setting of non-ischemic cardiomyopathies. Therefore the need for regenerative therapies for heart failure is becoming even more so important with an aging population as more patients are surviving their heart attacks and progressing on to develop heart failure (Akins 2002; Wu KH 2006; Li SC 2009).

2. Tissue Engineering

It is well known that the heart is one of the least regenerative organs in the human body (Wu KH 2006). Nevertheless, in recent years many scientists have envisioned the possibility of regeneration of the myocardium and reproduction of autologous heart tissue *in vitro* or *in vivo* (Zund G 1996; Shimizu T 2002; Leor J 2004; Shin M 2004; Zammaretti P 2004; Leor J 2005; Shaoping Zhong 2005; Ishii O 2006; Sekine H 2006; Zimmerman WH 2006). Heart tissue is composed of myocytes that are arranged in parallel and series so that contraction will generate a coordinated force to empty the ventricle. In brief, myocytes are elongated cells that are dedicated to contraction with most of the cellular cytoplasm occupied by actin/myosin chains, the structures responsible for contraction. They are connected by intercalated discs which conduct electrochemical potentials directly between the cytoplasms of the adjunct cells via gap junctions.

Gap junctions allow action potentials to spread directly between cells by depolarizing the heart via K^+/Na^+ exchange channels. This coordinated wave of electrical discharge coupled with mechanical contraction of the myocardium with each cardiac cycle is essential for normal pump function (Jansen JA 2009). Therefore, one can foresee that any regenerative therapy whether it is cell or tissue implantation will have to ensure appropriate incorporation of the transplanted tissue in a geometric fashion to ensure synchronous contraction with electrical and mechanical coupling. Additionally, the heart also utilizes a unique system for vascularization of the metabolically demanding tissues with a complex vascular bed (Chang AC 2006; Zimmerman WH 2006). This network would be essential for the survival, engraftment and functional incorporation of transplanted tissue.

Tissue engineering in general terms is a process that involves the reconstruction of tissue equivalents by combining biomaterials and living cells, to be used together in repair, maintenance and replacement and augmentation of native tissues or organs (Lanza RP 2000). The complexity of the human heart highlights the current hurdles that need to be overcome before the potential for regenerative therapies and tissue engineering for heart failure are fully realized. These challenges include, but are not limited to, the choice of cells/stem cells, processing, differentiation, engraftment, electrical and mechanical coupling of transplanted and native tissue and appropriate vascularization of the newly incorporated tissue, to name a few. The ideal myocardial construct should mimic the morphological, physiological and functional properties of the native cardiac muscle it intends to replace and needs to remain viable and functional long term after implantation.

Cardiovascular tissue engineering is an emerging field with an enormous potential for revolutionizing the next generation of heart failure therapies; we are witnessing the evolution of therapies from pharmaceuticals and device treatments to the era of biological therapies with cell and engineered tissue. This may eventually become a standard therapy for all heart failure patients post MI and also in the setting of non-ischemic cardiomyopathies to replace damaged and lost myocardium and prevent progression to end stage heart failure. For cell replacement therapy and tissue regeneration to be successful, it is essential to generate sufficient cells in a reliable and reproducible manner with the appropriate functional phenotype to replace the damaged tissue.
Not only do replacement cells or tissues need to be functional (for e.g. contraction in the case of cardiomyocytes, secretory in the case of pancreatic islet cells), these cells have to interact with their environment. This can be secretion of extracellular matrix, or maintenance of tissue and organ homeostasis through regulatory feedback mechanisms (Leor J 2004; Zammaretti P 2004; Leor J 2005; Zhong S 2005; Zimmerman WH 2006). Therefore, selecting the appropriate biomaterials and suitable cell source will be critical for the success of this strategy.

Cardiovascular tissue engineering is a materials-based approach and involves preformed three-dimensional (3D) scaffolds in the form of mesh, patch, or foam. This inert biocompatible material will then implanted with differentiated cells to form appropriate functional tissue to be transplanted in-vivo. This has been the focus of intense research in the cardiovascular tissue regeneration field over the past decade. (Zund G 1996; Shimizu T 2002; Leor J 2004; Shin M 2004; Zammaretti P 2004; Leor J 2005; Shaoping Zhong 2005; Ishii O 2006; Sekine H 2006; Zimmerman WH 2006).

The challenges of producing engineered tissues for *in vivo* use are considerable. The engineered tissue must provide an effective, durable, non-thrombogenic and non-immunogenic substitute that will display functional and morphological properties of the cardiovascular system. Furthermore, this material must possess repair and remodeling capabilities, and retain its viability after implantation (Langer R 1993; Lanza RP 2000; Atala A 2002).

In order for a potential cell source to represent a suitable candidate for the construction of engineered grafts, several criteria must be considered. The ideal cell should be autologous

and available in large quantities. If not autologous, the cells used must be non-immunogenic as to avoid the need for immunosuppression and all the associated difficulties and complications that are encountered in the transplant population. These range from the risk of graft rejection, increased infection and malignancy in patients on immunosuppression. It should also have the capacity to proliferate and differentiate *in vitro*, in a manner that can be reliably reproduced and controlled, and eventually automated for mass production to be practically viable in a clinical setting (Atala A 2002).
There are a number of limitations in the use of differentiated cells in tissue engineering. It is a challenge to generate sufficient numbers of a single cell type, to assemble the needed mixture of multiple cell phenotypes, and to maintain stable phenotypes as needed (Atala A 2002). Thus lies the promise of stem cells as an endless source that can be manipulated and combined with nanostructures and scaffolds to produce engineered tissue grafts to be used in clinical therapies.

Among different pharmaceutical and surgical treatments, the focus of emerging research is to use stem cells in order to heal or replace damaged cardiac tissues (Moffett BS 2006; Ruvinov E 2008; Segers VF 2008). Several kinds of stem cells are potentially useful because of their ability to self renew and differentiate into various types of cells in the body. Embryonic stem cells (ESCs) are capable of indefinite expansion and are pluripotent (able to differentiate into any cell type). However human embryonic stem cells (hESCs) although are immortal and pluripotent, they are burdened with ethical concerns due to their derivation from human embryos. Therefore their use has been very controversial and they have been limited to a very few lines only used in laboratory settings.

The ethical concerns surrounding these cells prevent them from being used in any clinical setting. Furthermore, issues with their immunogenecity, the risk of teratoma formation, the difficulties associated with their differentiation fate, and the ethical challenges arising from their embryonic origin have all excluded them as prime candidates for regenerative medicine (Petersen T 2007).

However, a recent breakthrough has completely revolutionized the field and taken it in a whole new direction. Using four transcription factors, Dr Yamanaka's group was able to reprogram mouse fibroblasts to a stem-cell like state creating induced pluripotent stem cells (iPS). Since then, many groups have been able to reprogram human, porcine, rat and murine somatic cells into these iPS cells using different approaches (Takahashi K 2006; Okita K 2007; Takahashi K 2007; Okita K 2008; Ezashi T 2009; Zhao XY 2009). These iPS cells have been differentiated into different tissue types and promise to revolutionize the regenerative cell and tissue replacement field.

Adult stem cells have been touted as a potential source of stem cells for cell and tissue replacement therapies although these cells present their own unique challenges. They have been found to vary in quality depending on the age and health of the donor/patient and differentiation is often restricted to the origin lineage of the cell source (multipotent) (Smith S 2007). An important consideration is the expansion potential of the adult stem cells to be considered as a viable source for regenerative cell therapy potential. Unlike ESCs that have an almost unlimited expansion capability, adult stem cells may be capable of only a limited

number of doubling cycles. Therefore, candidates that are able to provide an abundant number of cells that would be required for cell and tissue replacement therapies maybe embryonic stem cells, bone marrow derived cells, bone marrow derived mesenchymal stem cells, endothelia progenitor cells, skeletal myoblasts, adipose derived stem cells and induced pluripotent stem cells.
This chapter will focus on adipose derived stem cells (ASCs) and induced pluripotent stem cells (iPS).

3. Adipose Derived Stem Cells

For the production of clinically useful tissue-engineered constructs to replace cardiovascular structures, it is critical to identify a suitable cell source that is autologous and capable of differentiating into the desired cell types. Adipose tissue represents an easily accessible and abundant source of cells. Furthermore, this source represents a potential adult stem cell reservoir for each individual (Aust L 2004). In recent years, interest has rapidly grown in the developmental plasticity and therapeutic potential of stromal cells isolated from human subcutaneous adipose tissue.

Adipose tissue represents an abundant, practical, and appealing source of donor tissue for autologous cell replacement (Planat-Benard V 2004a; Planat-Benard V 2004b; Katz AJ 2005). Adipose tissue is of mesodermic origin, consisting of mature adipocytes and the stromal vascular fraction (SVF). SVF is a heterogeneous cell population, consisting of vascular cells (endothelial cells (ECs) and smooth muscle cells (SMCs)), blood cells, and a fibroblast-like multipotential stem cell population, termed adipose-derived stem cells (ASCs).
These cells can be isolated in large numbers with minimally invasive techniques from liposuctions and grown easily under standard tissue culture conditions (Zuk PA 2001; Zuk PA 2002), and therefore represent an excellent abundant stem cell source with a high therapeutic potential. Since its initial characterization, several groups have demonstrated the ASC population of stem cells within the SVF of subcutaneous adipose tissue displays multilineage developmental plasticity *in vitro* and *in vivo*.

It has been shown that adult stem cells from white adipose tissues can differentiate into multiple cell phenotypes, including the adipocyte, chondrocyte, epithelial, hematopoietic, hepatocyte, neuronal, myogenic, and osteoblast lineages (Halvorsen YC 2000; Halvorsen YD 2001; Zuk PA 2001; Erickson GR 2002; Mizuno H 2002; Safford KM 2002; Zuk PA 2002; Cousin B 2003; Gimble J 2003a; Gimble JM 2003b; Kang SK 2003; Kim DH 2003; Rangappa S 2003; Miranville A 2004; Planat-Benard V 2004a; Planat-Benard V 2004b; Rodriguez LV 2006).
Research efforts towards understanding the nature of how ASCs differentiate into cells of the cardiovascular lineage, including ECs and SMCs, have become more intensive in the past years. Recent studies have shown that ASCs are capable of differentiating into either SMCs (Lee WC 2006; Rodriguez LV 2006) or cells of an EC phenotype (Planat-Benard V 2004; Dimuzio P 2006; Wosnitza M 2007). However, the differentiation protocols employed in those studies are rather diverse, using complex cell culture media that are supplemented with various growth factors. Moreover, the culture time periods that were reported to be necessary for a successful differentiation of ASCs into cardiac cells were rather exhaustive,

making this an unpractical and unfavorable option for potential clinical applications. However, there has yet to be significant research into the relationship between patient's age, gender, comorbidities and the differentiation capabilities of these adipose derived cells.

We have focused on the differentiation of hASCs towards the endothelial and SMC lineage. We have been able to identify specific parameters that are crucial for an efficient and easily reproducible differentiation of human ASCs into ECs and SMCs within 14 days of *in vitro* culture. We found that hASCs, which were cultured in endothelial specific growth medium-2 (EGM-2), exhibited morphological features of mature ECs and expressed EC-specific markers including CD31, CD144 and vWF (Heydarkhan-Hagvall S 2008).
This differentiation pattern appeared to be specific to EGM-2-treated hASCs, cultured in a high-density environment. It has been reported that cell-cell junctions between ECs through CD31 and CD144 are critical for the establishment of a primary vascular network (Dejana E 1995; Baldwin HS 1996; Dejana 1996; Drake CJ 1998).
The failure of hASCs that were cultured in Dulbecco's modified Eagle's medium-20% (DMEM-20%) FBS to form such tubular network may, in part, be due to the absence of these necessary cell-to-cell contacts. Our immunocytochemistry and fluorescence-activated cell sorter (FACS) analyses demonstrated the presence of both immature and mature EC markers, such as CD34, Flk-1/KDR, CD144, CD31 and vWF, in the specifically induced hASCs (Heydarkhan-Hagvall S 2008).

Previous studies have identified three markers, CD133, CD34 and Flk-1/KDR, in early endothelial progenitor cells. In addition, mature ECs are known to express high levels of Flk-1/KDR, CD31, CD144, and vWF, suggesting that our EGM-2-induced hASCs may be assuming a more mature EC phenotype. To date, it is not completely understood as to when an endothelial progenitor cell turns into mature, fully differentiated ECs *in vivo*. One possibility could be the loss of CD133 and a parallel or subsequent expression of vWF in conjunction with the appearance of other endothelial characteristics (Hristov M 2003; Urbich C 2004). An interesting observation was that the differentiation capacity of hASCs towards ECs was significantly diminished when the cells were passaged, whereas the age of the donor was not a critical factor.
In contrast, when hASCs were cultured for 14 days in DMEM-20% FBS they expressed SMC-specific markers including SM myosin, h-caldesmon, basic calponin and SM-α-actin a phenomenon that was independent of passage number, yet affected by the donor age (Heydarkhan-Hagvall S 2008). As the primary function of mature SMC is contraction, the complement of contractile, structural, and regulatory proteins expressed in fully differentiated SMC provides markers of differentiation status (Kuro-o M 1989; Sobue K 1999). These include SM myosin, h-caldesmon, basic calponin, and SM-α-actin. SM-α-actin is an important protein for SMCs structure and contraction, and it is upregulated with differentiation. Indeed, SM-α-actin has been used as a marker of early SMC differentiation, followed by calponin, h-caldesmon and SM myosin (Owens GK 1995; Hungerford JE 1996; Katoh Y 1996; Thyberg J 1996; Hungerford JE 1999).
It is well known that SMCs grown in vitro can undergo a phenotypic cell transition from the normally quiescent 'contractile' status observed *in vivo* to a proliferative-secretory state (Thyberg J 1996; Opitz F 2004). The general feature of cultured SMCs is the downregulation of SM myosin expression, particularly of SM2 concomitant with upregulation of nonmuscle

myosin variants MyHC-A pla1, MyHC-A, and MyHC-B (Somlyo AP 1993). These tendencies depend, however, on cellular density, serum concentration, and substrate for attachment. Myosin gene expression can be regulated during early development (effect on the differentiation phase of the SMC lineage program) or in adulthood (alteration of the stability of the fully differentiated SMC phenotype) (Zanellato AMC 1990; Somlyo AP 1993). The general feature of cultured SMCs is the downregulation of SM myosin expression, particularly of SM2 concomitant with upregulation of non-muscle myosin variants MyHC-A pla1, MyHC-A, and MyHC-B (Somlyo AP 1993). These tendencies depend, however, on cellular density, serum concentration, and substrate for attachment. Myosin gene expression can be regulated during early development (effect on the differentiation phase of the SMC lineage program) or in adulthood (alteration of the stability of the fully differentiated SMC phenotype) (Zanellato AMC 1990; Somlyo AP 1993). High levels of SM-α-actin and basic calponin in particular were observed in the present study, with lower levels of SM myosin and h-caldesmon-expressing cells. Rodriguez et al. (Rodriguez LV 2006) demonstrated an optimal smooth muscle differentiation, phenotypically and functionally, when hASCs were cultured in specific smooth muscle-inductive medium after 6 weeks. However, our data have shown that a prolonged culture time did not increase the number of SMC marker expressing cells, since a plateau was reached after 14 days of culture.

4. Induced Pluripotent Stem Cells

One of the most exciting and revolutionary breakthroughs in stem cell research has been the induction of pluripotent stem cells from mature somatic cells. It has been shown that four factors present in ESC are sufficient to re-induce a pluripotent state in somatic cells. The reprogramming of somatic cells to a pluripotent state can be achieved by simple retroviral overexpression of specific transcription factors, resulting in induced pluripotent stem (iPS) cells that are almost indistinguishable from ESC (Okita K 2007; Mauritz C 2008; Narazaki G 2008; Winkler ME 2008).

Transduction of fibroblasts with only four transcription factors, Oct4, Sox2, cMyc and Klf4, could dedifferentiate the fibroblasts to cells with almost all features of ESC. The key transcription factors needed for reprogramming appear to be Sox2 and Oct4, whereas the other transcription factor used allow for increasing the efficiency of reprogramming likely by opening up the closed chromatin typical of somatic cells ESC (Mitsui K 2003; Silva J 2006; Takahashi K 2006; Takahashi K 2007; Yu J 2007; Park IH 2008).

Initially, reprogramming was accomplished using integrating retroviral or lentiviral vectors, however these vectors carry the risk of insertional mutagenesis, which will make this method of reprogramming unacceptable for use clinically in patients due to increased risk of tumor formation. However, the problem of using retroviral integrating vectors has been overcome as reprogramming has been achieved by many groups using different modalities as a series of very recent studies demonstrate that this can also be achieved using short term overexpression of the transcription factor using plasmids, nonintegrating adenoviral vectors, or using transposons that are subsequently removed. In contrast to reprogramming by somatic cell nuclear transfer (SCNT) or ESC fusion which were the original methods of inducing pluripotency and were very laborious, technically difficult and time consuming, reprogramming via defined transcription factor requires 3 to 4 weeks, suggesting that many

more factors may take part in the very quick reprogramming seen by SCNT or fusion (Yu J 2007; Okita K 2008; Shi Y 2008a; Stadtfeld M 2008a; Shi Y 2008b; Woltjen K 2009; Yu J 2009).

The ability of iPS cells to differentiate into all somatic cell typeshas attracted much interest in the field of regenerative medicine and has been a topic of intense research in recent years (Dai W 2006). This interest has become even more relevant from a clinical practical perspective as these reprogrammed fibroblasts exhibit growth and differentiation characteristics comparable to those of murine ES cells. Ease of generation and lack of immunologic and ethical restrictions will likely make iPS cells a highly valuable cell source for applications in regenerative medicine.

Given the appropriate extracellular signals, murine iPS cells differentiate with high efficiency into multipotent mesodermal progenitor cells that possess the potential to differentiate into functional cells of the cardiovascular and hematopoietic lineages (Schenke-Layland K 2008). When exposed to Collagen IV (ColIV), ESC and iPS cells differentiated into cells that showed expression of genes associated with early mesodermal, cardiovascular, and hematopoietic cells.
A cell capable of differentiating into all cardiovascular cell types has a theoretical advantage for more complete tissue regeneration over transplanting cardiomyocytes alone, as has been demonstrated for ESC derivatives (Dai W 2006). Transplanting partially differentiated cardiovascular progenitor cells, such as the Flk1-positive cells which are committed to a cardiovascular lineage will not be associated with the risk of tumor formation as seen with transplanting undifferentiated ES cells into the heart (Behfar A 2007). Flk1-positive progenitor cells have been identified in both iPS cell-derived embryonic bodies (EBs) and ColIV-differentiated iPS cells, most likely representing a population of multipotent mesodermal progenitor cells (Park C 2005; Kattman SJ 2006; Moretti A 2006; Wu SM 2006). To confirm that those Flk1-expressing cells were capable of generating all cardiovascular cell types, ColIV differentiated Flk1-positive cells have been isolated and been exposed to cardiac, smooth muscle, and endothelial cell-specific differentiation conditions. This resulted in the production of spontaneously beating cell clusters and cells expressing cardiac markers as well as hallmark morphological characteristics of mature cardiomyocytes, including the typical cross-striation and generation of Ca^{2+} (Schenke-Layland K 2008).
Gene expression analysis, immunocytochemistry, contractility, and *in vitro* tube formation assays, as well as acLDL uptake tests, further revealed the successful differentiation of iPS cell-derived Flk1-progenitor cells into functional SMC and EC, findings that were similar to those previously reported for murine ES cells (Nishikawa SI 1998; Yamashita J 2000; McCloskey KE 2006). Although iPS cells contributed to mature cardiovascular cells *in vivo* as well, it will be important to determine in future experiments whether transplanted iPS cells can also integrate and differentiate into adult myocardium. In addition, co-culture of the Flk1-positive cells with OP9-GFP stromal cells in hematopoietic cytokine-containing culture medium conferred differentiation into hematopoietic progenitor cells that expressed c-kit, CD41, and the pan-hematopoietic marker CD45 (Mikkola HK 2006).
Furthermore, these ES and iPS cell-derived hematopoietic progenitors demonstrated a multilineage myeloerythroid differentiation potential. Although ColIV-differentiated iPS cell-derived Flk1-positive progenitor cells had properties comparable to those of the ES cell-derived progenitors, differences did exist (Yamashita J 2000; Kattman SJ 2006; McCloskey

KE 2006; Moretti A 2006; Wu SM 2006). Flk1-positive progenitor cells isolated from the ColIV-exposed cultures also possessed hematopoietic differentiation potential when cultured on OP9 stromal cells or in methylcellulose. In addition, Flk1-positive cells were more frequent in ColIV-differentiated iPS cell cultures compared with ES cell cultures, but whether this is a general property of iPS cells or is specific to the 2D4 iPS cell line will require further study and comparison of multiple lines (Schenke-Layland K 2008).

5. Discussion and Conclusion

The use of living cells as a therapeutic option presents several challenges including identification of a suitable source, development of adequate derivation, maintenance and differentiation methods, and very importantly proof of safety and efficacy. One of the major issues for cardiovascular tissue engineering is determining the ideal cell type for use in regenerative therapies.Many clinical trials have used bone marrow derived mononuclear cells (BM-MNC) (Schächinger V 2006). These clinical trials have not shown any significant cardiomyocyte regeneration and the results have been mixed at best with no robust improvement in cardiac function (Coombs 2008). However, this trial and others have provided a proof of concept that intracoronary or intramyocardial transplant of autologous adult stem cells is safe with out any evidence of increased mortality in the treated patients. The next generation of clinical studies will need to demonstrate robust cardiomyocyte regeneration, definite improvement of cardiac pump function, and ultimately improved patient survival as the ultimate goal. The availability of the proposed cell type for regenerative medicine and tissue engineering in sufficient and relatively easily derivable quantities is also critical. To date, engineered tissue constructs containing adult and stem cells have exhibited problems with physical properties, maintenance of cell phenotypes and the host immune response to the engrafted construct. Ideally, the cells used for tissue engineering should have the capacity to proliferate and differentiate in vivo in a manner that can be reproducibly controlled and predicted (Atala A 2002). Due to the high number of cells that is needed for culturing, isolation and expansion require invasive procedures it remains a challenge to generate sufficient numbers of a single cell type, to assemble the needed mixture of multiple cell phenotypes, and to maintain stable phenotypes as needed (Atala A 2002). Furthermore, this process will need to be automated to mass produce sufficient amounts of cells and tissue constructs for clinical therapies on a wide scale.

This discussion also highlights the ushering in of a new era of personalized medicine. One of the possibilities for cell therapies is patient-specific iPS cells generated from his/her own somatic cells to be used to treat that person. These designer iPS cells will be differentiated into the desired tissue types and transplanted in an autologous manner to avoid immune rejection. Although pluripotent hESCs or hiPS cells are non-immunogenic, they loose this characteristic as they become more differentiated with increased risk of immune mediated rejection if transplanted into a non-compatible patient. ASCs derived from patients may be a potential source to use to either differentiate into desired cells and re-transplant into the patient or develop and differentiate iPS cells that would be used in an autologous manner to ensure immunocompatibility as it has been shown that ASCs are much more easily induced into hiPS cells compared to fibroblasts (Sun N 2009).

The isolation of hESCs more than two decades ago was hailed as the beginning of the end of many diseases; but as we can see this potential many years later has not been realized. It was hoped that creating differentiated cells from hESCs might lead to tissue replacement treatments for diseases such as Parkinson's, diabetes, cardiovascular diseases etc. However, the ethical concerns with the use of hESCs due to their origin from human embryos have limited their use to very restricted research with no translational applications to date.
Another obstacle to the use of ESC and iPS derived cells and tissues is the risk of teratoma formation in patients post cell/tissue transplant. The first ever clinical trial to use hESCs was to be done in spinal cord injury patients and was to be launched in 2009 by the biotechnology firm Geron and had FDA approval; before any patients were enrolled, it was put on hold by the FDA due to concerns with results from animal studies regarding higher than acceptable rates of tumor formation in the animal models post ESC transplants.
Moreover, other concerns with ESC based therapies is allogenicity of ESC derived cells which would necessitate life long immunosuppressive therapy so the donor would not reject the transplanted cells or tissue grafts. This is not a favorable option as immunosuppression has many undesirable side effects, such as increased risk of infection and malignancy, and all the side effects of these medications. Thus lies the promise and potential of cells or tissue grafts derived from autologous derived ASCs or patient derived iPS cells, as immunocompatibilty with the host would negate the need for immunosuppression. Although generating patient specific stem cells is, at the current costs to generate good manufacturing processing (GMP) grade cells, not financially tenable, it is not unreasonable to speculate that costs, as with any new technology, will decrease with time making personalized stem cell therapy for at least some diseases possible. One other possibility would be HLA matched banks of hiPs that will be available to use for clinical use that can be matched to the patient of interest.

One other potential for the use of autologous iPS cells lies in the fact that they would encode for the same genetic defects of the patient whether inherited or caused by sporadic genetic mutations. This ability to generate cells with pluripotent characteristics that differentiate into most cell types will also make it possible to generate *in vitro* models of human disease (Dimos JT 2008; Park IH 2008; Wernig M 2008). This presents a great opportunity, for the first time, to study human disease models in vitro and drug discovery directly on diseased or normal human cells derived from iPS cells. Already iPS cells from patient with Parkinson's disease have been generated and differentiated into neurons (Park IH 2008). The other exciting possible application of patient derived iPS cells is the potential for repair of disease causing genetic defects by *in situ* repair of the defect using homologous recombination and re-transplant of these modified cells back into the patient. This has been demonstrated in a mouse model of sickle cell anemia with cure of the mouse from the disease (Hanna J 2007).

Although a number of differentiation protocols induce lineage specification *in vitro*, engraftment of the pluripotent stem cell-derived differentiated cells *in vivo* has been disappointing (Kyba M 2002; Rideout WM 3rd 2002). The availability of pluripotent stem cells will however in the near future influence medicine far and beyond the realm of tissue engineering and replacement. For instance, availability of cells that can generate many differentiated cell types, may lead to the development of protein or small molecule drugs

that influence differentiation not only from pluripotent stem cells but also of multipotent stem cells residing in different tissues. The ability to generate cells with pluripotent characteristics that differentiate in most cell types will also make it possible to generate *in vitro* models of human disease (Dimos JT 2008; Park IH 2008; Wernig M 2008). The identification of optimized protocols for the differentiation of ESCs iPS cells into multiple functional cell types *in vitro* and their proper long term engraftment and fate tracking *in vivo* will be the next generation of challenges once the issue of reprogramming and optimal sources of cells is resolved.

Moreover, the risk of oncogenic events caused by the use of potent oncogenes to induce reprogramming to pluripotency and by the random integration of delivery vectors into the genome is a major problem that needs to be overcome before translating iPS cell technology into the clinic. (Marson A 2008; Huangfu D 2008a; Shi Y 2008a; Huangfu D 2008b; Shi Y 2008b) Murine and human iPS cells have recently been derived by using nonintegrative transient expression strategies of the reprogramming factors (Okita K 2008; Okita K 2008; Stadtfeld M 2008a; Stadtfeld M 2008b; Stadtfeld M 2008c).
Regardless of these uncertainties, direct reprogramming of somatic cells to generate patient-matched pluripotent stem cells has the potential to address many of the current limitations and could revolutionize the treatment of many diseases. As with other non-embryonic derived tissue sources, iPS cells escape all of the ethical issues surrounding ESC therapy. The development of efficient, reliable, and easily reproducible differentiation protocols for generating human iPS cell-derived cardiovascular and hematopoietic progenitor cells will facilitate the development of patient-tailored cardiovascular and hematopoietic regenerative therapies as the next generation of approach to these diseases.

It has been shown that hASCs differentiate into different cell types (Halvorsen YC 2000; Zuk PA 2001; Zuk PA 2002; Gimble J 2003a; Gimble J 2003b; Planat-Benard V 2004a; Planat-Benard V 2004b; Zhu Y 2008) and recent work has shown that these hASCs can be induced much more easily into iPS cells than terminally differentiated fibroblasts. This is quite exciting and clinically relevant as hASC-derived hiPS cells can be differentiated into any type of desired cell type (Sun N 2009). ASCs can be a potential alternative cell source for producing iPS cells as they are widely available and easily obtainable from patients.
The expression of the transcription factors that have been used in reprogramming of fibroblasts to iPS is normally very low in adult cells such as fibroblasts. On the other hand these stem cell-related transcription factors, Nanog, Oct-4, Sox-2, and Rex-1 are positively expressed in ASCs (Zhu Y 2008); this makes ASCs easier to reprogram back to an earlier state than fibroblasts since they are not as far along on the differentiation pathway.
The gene expression profiles of the ASCs could be used to identify a subpopulation that is the least differentiated and most easily reprogrammed to pluripoteny (Sun N 2009). The recent advances in iPS cell identification and differentiation and eventual transplant of these iPS derived cells and tissues has ushered in the new era of biological therapeutics to complement the current pharmaceutical modalities. In addition, further development of iPS cell technology may provide a more substantial and dynamic stem cell population available to overcome current stem cell shortages and will very likely be a standard of tissue engineering and patient therapy in the not so distant future.

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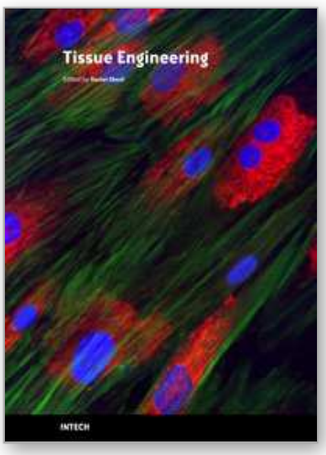

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The Tissue Engineering approach has major advantages over traditional organ transplantation and circumvents the problem of organ shortage. Tissues that closely match the patient's needs can be reconstructed from readily available biopsies and subsequently be implanted with minimal or no immunogenicity. This eventually conquers several limitations encountered in tissue transplantation approaches. This book serves as a good starting point for anyone interested in the application of Tissue Engineering. It offers a colorful mix of topics, which explain the obstacles and possible solutions for TE applications.

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