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Cell Releasing System Using Light Responsive Photovoltaic Devices

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Dedication

I would like to dedicate this work to my parents, my siblings, my wife, Zobeda Khathun, and my son Faiaz and Arryan. My family stood beside me through my thick and thin during the completion of my study. Without their cordial love, patience, and inspiration, I would not be able to finish this work and my PhD program.

CELL RELEASING SYSTEM USING LIGHT RESPONSIVE PHOTOVOLTAIC DEVICES

by

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Abstract

Tissue and organ regeneration via transplantation of cell bodies in-situ has become an emerging strategy in regenerative medicine. Injected or implanted cells must be available in large enough numbers and suitable delivery methods must be found especially when implantation into the affected tissue is not possible. In addition, better understanding of how to mitigate the inflammatory reactions and how to degrade the cell carriers are needed. Current cell-based therapies are limited to injection or implantation of cell suspensions or implantation of cell sheets. Development of alternative systems to obtain cell sheets or other functional cellular structures are required. We are investigating light treatment as an efficient, safe, and repeatable method to release cells, cellular aggregates, structures or sheets for implantation in regenerative medicine.

We hypothesize that cells cultured on the surfaces of a photovoltaic element may be released by applying visible radiation to the element. The light will induce a surface charge on the element causing cells to be released by electrostatic repulsion. Herein, we conducted a study that examines the amount and viability of myoblasts cells released from a photovoltaic element upon light exposure. We investigated (i) the biocompatibility of PV devices, (ii) the utilization of PV devices as cell substrate iii) the release of attached cells from PV surfaces upon light stimulation, and (iv) the progressive release of proliferated cells from PV devices. C2C12 myoblast cells were cultured on sterile silicon based photovoltaic elements with n-type surface under typical cell culture conditions. Upon confluence, the elements were exposed to low power visible lights for 1-2 hours. Cells in the supernatant and those attached were collected and counted. In addition live/dead assays and DAPI staining were performed on the released cells. Cells released by light exposure were re-seeded for further culture. The results showed that approximately 40% of attached cells could be released from the element upon a light exposure. This strategy may be used to release cells or cellular structures for eventual use in regenerative medicine.

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Chapter 1: Introduction

Cell based therapy is the transplantation of cells aiming to prevent organ failures or malfunctions. Current cell-based therapies are divided into scaffold-based, and scaffold-free approaches. Scaffold-free approaches include injections of cell suspensions or implantations of cell sheets. Cell therapies may eventually lead to, artificial or scaffold based organs as a substitute for organ transplantation [1]. The primary limitation of cell therapies is the inadequate delivery of nutrients, which not only harms the transplanted cells but may also causes progressive deterioration of the host tissue. For scaffold based tissue engineering, a major complicating factor is the inflammatory reactions that take place upon their implantation and biodegradation of structural materials. Degradation mechanism and byproducts can cause harm to the seeded cells within the scaffolds limiting the cell mass [2]. Consequently, larger organs such as the heart, liver, bladder or kidney, have not been re-generated with this method.

Over the last decades, there has been great progress in cell based therapies, especially in the area of the identification, isolation, and bioprocessing of cells for reconstruction of damaged organs. One of the key challenges facing the field of cell therapy is to translate in vitro cultures into an implant that remains functional. Cell therapies often involve injection of cell suspensions with proper medium into damaged or diseased areas or directly into the systemic blood flow as shown in (Figure 1.1). Various types of cells including neural stem cells [3], myoblast populations [4], and a variety of bone marrow derived cells [5] have been transplanted by injection and were studied with respect to contributing in the restoration of diseased or damaged tissues. In some cases, the cells attached to target site and some other studies found that injected cells connected to the existing cell circuitry and improved organ functions [6]. A second

mechanism has been proposed, which hypothesizes that the injected cells act as reservoirs and secret growth factors thus participating indirectly in the regeneration of tissues or organs [7-9].

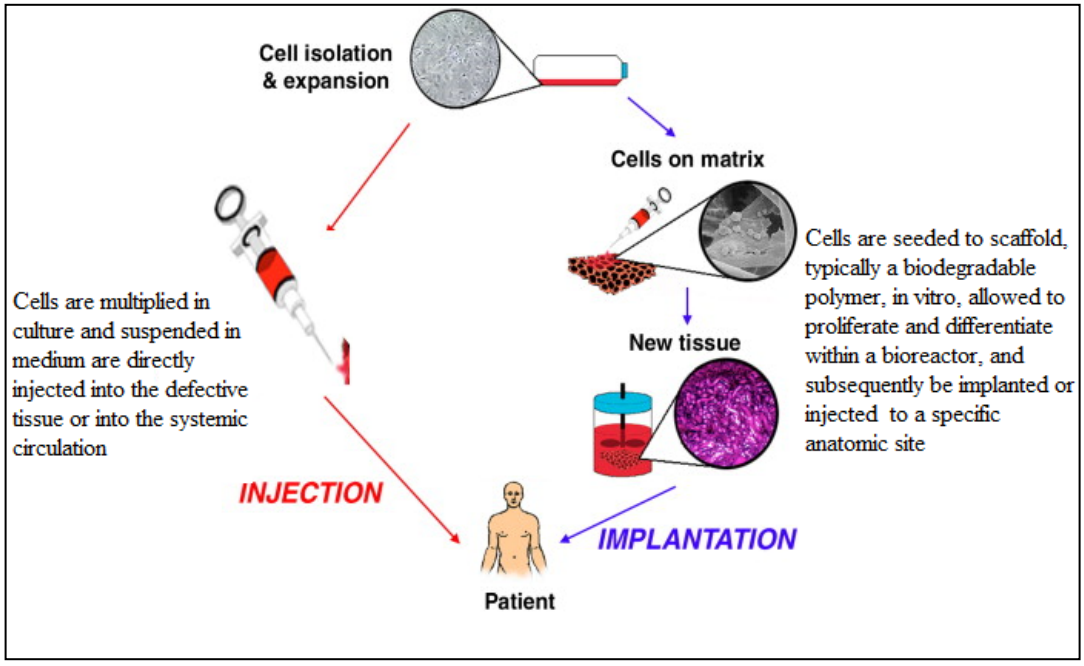

Figure 1.1: Current Strategies for Cell Transplantation [10]

Over the past few decades, biomaterials, including several hydrogels, have been widely used as carriers for cell delivery or vehicles to promote tissue regeneration [10-12] in cell encapsulation/delivery systems. Some studies have reported uniform spatial distribution and localization of cells via direct needle injection at the required site of delivery [9], [14]. Others have found that systemic administration or targeted delivery can also be performed by infusion followed by homing [15]. Local infusion and direct injection have the advantage of being extended from the body surface or through a catheter; however, cells experience small targeting efficiencies as a high quantity of administered cells are washed out from the blood stream.

Because of the low targeting efficiencies, this approach has not yet been translated into regenerative medicine. More efficient delivery and controled release of therapeutic cells are urgently needed. Studies that look at cell delivery as a function of time, path of administration, cell density, viability, delivery medium, and accurate spatial distribution to the host site are necessary [16].

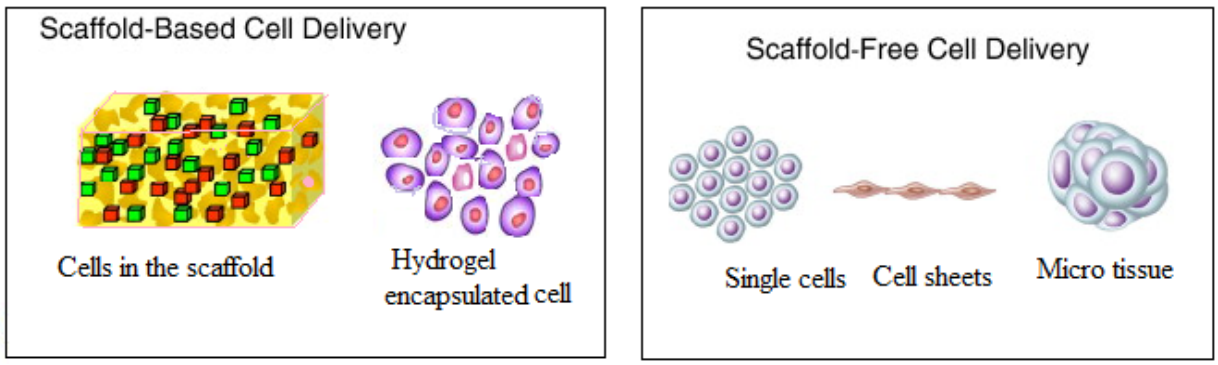

Figure 1.2: Cell-delivery formats can be either scaffold-based or scaffold-free [17].

Two different approaches are employed to carry cells into tissues: (1) transplants containing cells encapsulated with hydrogels or cells embedded on a scaffold and (2) transplanting cell sheets and single cells. The former is also known as a scaffold based approach and the latter as a scaffold free approach.

Largely, the selection of the approach chosen depends on the type of diseases needing cell-based therapy and what kinds of cells are to be used as therapeutic agents. In single cell delivery, cell seeding efficiencies, and cell viability are crucial in determining the outcome of the treatment. Seeding efficacies fluctuate from 0% to 90%, depending on the type of cell, cell quantity, and methodology used. Cell encapsulation and number of cells to be delivered are also important parameters, because they determine mechanical properties and reduce pore size, and

allow greater retention of proteoglycans [18]. Lastly, the sources of the injected cells are an important factor because their ease of isolation, in vitro expansion, handling, self-renewal capacity, and capability to differentiate into various cell types will affect the outcomes.

For scaffold-based approaches, cells are nested in scaffolding materials that provide biological and mechanical support. A variety of natural extra cellular matrices are used as cell carriers or vehicles. These include type I collagen and fibrin due to their inherent cell attachment capabilities. Interactions of the cells with the scaffold materials play a significant role in the cell attachment and differentiation [19]. For example, it has been shown that the firmness of the adhesion substrate affects the *in vitro* differentiation of mature tissue-derived progenitor cells [20]. Cell adhesion, therefore, impacts the capacity of forming new tissue structures and contribute in regeneration of injured tissues [19]. In addition to collagen and fibrin, other hydrogels and various stimulus responsive biomaterials are used for cell encapsulation. Encapsulated cells are used in a wide range of application in the cell therapy [21]. However, assembly of encapsulated cells is a tedious process and the design of proper delivery systems is required. Commonly used stimulators or triggers holding encapsulated cells include temperature for collagen [22] and Matrigel [23], pH change for PuraMatrix [24], leucine-zipper systems [25], boosting of cation concentration for alginate [26] and peptide amphiphiles [27]. As cells are highly sensitive to external stimulus and abrupt changes in physiological state, these triggers can be harmful to the encapsulated cells and associated protein. In some cases the intensity of external stimulation can cause severe toxic immunogenic or mutagenic reactions.

Both encapsulated and scaffold free cells delivered via intravenous injection are distributed systemically, and merely a small portion of the injected cell dose reaches the intended destination for regeneration [28]. Additionally, even when a small portion of the cells reach the

target tissues, their restorative or healing activities can be extremely short-lasting and frequent repeated dosing can be required.

A significant challenge to the successful implementation of cell therapies is the integration of delivered cells with the host. Cells need to integrate quickly with the host's lymphatic, vascular, and nervous systems. Rapid attachment and adjustment of cells must occur to avoid anoikis or programmed cell death. Transplanted cells will die quickly if they are not delivered to the targeted side or within 100 μm from tissue vasculature. However, several studies have shown that survival can be improved by over expression of pro angiogenic factors [8], [29-31].

Although cellular therapy has great deal of potential, there are still the following noteworthy challenges to overcome:

- Designing cell specific biomaterials for scaffold-based regenerative treatments
- Noninvasive administration or transplantation with high dose of cells and therapeutic efficacy
- Most fitting cell type and timing of treatment
- Design new delivery systems that integrate various cues for cell homing
- Identifying suitable biocompatible and biodegradable materials for anchorage dependent cells as cell carrier or vehicle
- Cell release from the cell-substrate with external stimulation

Anchorage dependent mammalian cells are greatly affected by cell–substrate interactions [32]. Various hydrogels have been used for biomedical applications, but biocompatible silicon substrate exhibits a unique potential in adherence and viability of cells [33] and has been very

little explored until the present time. In this study, we test whether biocompatible silicon based Photovoltaic (PV) devices that serve as cell culture substrates can be used as cell delivery vehicle or carrier. Monocrystalline silicon cell culturing surface charges can be manipulated with light to reveal mechanistic information on action potential cell fate such as linkage, release and migration. Photovoltaic cells are devices that convert light into voltage as well as induces charge transport by the photovoltaic effect [34]. Therefore, silicon based PVs have the potential to serve as a new cell vehicle or carrier to culture cells, and releases them upon external photo stimulation. The ultimate goal of this study is to develop a cell release mechanism based on light activated photovoltaic devices. We are investigating if the charged anchorage dependent cells can be released from the PV device upon light exposure.

1.1 Cell therapy as therapeutics

Existing therapeutic approaches to healthcare and medicine have been very efficacious in developing new treatments for various critical and chronic disorders or infections. However, numerous patients are suffering without proper treatment from lingering illness. Successful regenerative treatments and cell therapies could greatly improve the diseases and conditions of innumerable patients by enhancing the quality of life. Considering the root cause syndrome at cellular level, cell therapies have the strong effect to cure or reduce the ailment of some chronic and critical conditions including trauma, heart disease, progressive neurological disorder, and autoimmune diseases. At present, 13% of US population are older than sixty five years of age and this percentage is going to jump on 19% in next two decades [35]. As a result diseases such as cancer, diabetes, heart disease, stroke, pulmonary disease will remain high in prevalence. Furthermore, older people are also susceptible to degenerative diseases such as Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and Huntington's

disease (HD). The treatment of those chronic and neural diseases is that of care either in a clinical setting or at home to relieve symptoms, rather than a cure. This will burden the US healthcare system with increased healthcare spending that is predicted to rise sharply both on a per capita basis and as a percentage of the US federal budget.

1.2 Justification of the work

The major outcome of regenerative medicine is to directly improve health condition by wound repairing or replacement and corresponding economic savings. Despite the huge potential, few regenerative treatments or products are in the market. Two of the major hurdles are the estimated one billion dollar cost, and regulatory compliance with the Food and Drug Administration (FDA). Therefore, most challenging aspects of developing novel therapy is that it has to be economically viable, effective, safe as well as easy to produce. Existing regenerative treatments for organ repair or replacement is not only in short supply, there are also processing, economical, medical, ethical, pre-operative and postoperative issues.

In the current cell delivery methods, cell loading, cell release, cell dose, timing of administration and cell encapsulation are an intricate manufacturing process. The cells used for regenerative therapy may die during cell processing and pose a challenge to interact with substrate materials. Furthermore, there are other limitations that include optimal delivery methods, insufficient localization, cell retention, cell survival, and lack of a matrix for cell attachment that can result in cell death [36]. To achieve a simple and safe process for cell-based therapies, new tools and technologies are needed. Our novel study, development of a light stimulated cell release mechanism from PV devices, might be able to resolve current cell delivery obstacles. We strongly believe that current noninvasive cell release method will lead to establish a robust and viable system for cell release with better cell survival and engraftment.

References

1. Tabata, Y., Significance of release technology in tissue engineering. Drug Discov Today, 2005. **10**(23-24): p. 1639-46.
2. Sung, H.-J., et al., The effect of scaffold degradation rate on three-dimensional cell growth and angiogenesis. Biomaterials, 2004. **25**(26): p. 5735-42.
3. Johansson, C.B., et al., Identification of a neural stem cell in the adult mammalian central nervous system. Cell, 1999. **96**(1): p. 25-34.
4. Partridge, T.A., et al., Conversion of mdx myofibres from dystrophin-negative to -positive by injection of normal myoblasts. Nature, 1989. **337**(6203): p. 176-9.
5. Krause, D.S., et al., Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell, 2001. **105**(3): p. 369-77.
6. Hou, D., et al., Radiolabeled cell distribution after intramyocardial, intracoronary, and interstitial retrograde coronary venous delivery: implications for current clinical trials. Circulation, 2005. **112**(9 Suppl): p. I150-6.
7. Rehman, J., et al., Peripheral blood "endothelial progenitor cells" are derived from monocyte/macrophages and secrete angiogenic growth factors. Circulation, 2003. **107**(8): p. 1164-9.
8. Rehman, J., et al., Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. Circulation, 2004. **109**(10): p. 1292-8.
10. Mooney, D.J. and H. Vandenburgh, Cell delivery mechanisms for tissue repair. Cell Stem Cell, 2008. **2**(3): p. 205-13.
11. Drury, J.L. and D.J. Mooney, Hydrogels for tissue engineering: scaffold design variables and applications. Biomaterials, 2003. **24**(24): p. 4337-51.

12. Mellott, M.B., K. Searcy, and M.V. Pishko, Release of protein from highly cross-linked hydrogels of poly(ethylene glycol) diacrylate fabricated by UV polymerization. Biomaterials, 2001. **22**(9): p. 929-41.

13. Peppas, N.A. and W. Leobandung, Stimuli-sensitive hydrogels: ideal carriers for chronobiology and chronotherapy. Journal of biomaterials science Polymer edition, 2004. **15**(2): p. 125-44.

14. Mangi, A.A., et al., Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. Nature medicine, 2003. **9**(9): p. 1195-201.

15. Al Mheid, I. and A.A. Quyyumi, Cell therapy in peripheral arterial disease. Angiology, 2008. **59**(6): p. 705-16.

16. Gill, S.S., et al., Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease. Nat Med, 2003. **9**(5): p. 589-95.

17. Kelm, J.M. and M. Fussenegger, Scaffold-free cell delivery for use in regenerative medicine. Advanced drug delivery reviews, 2010. **62**(7-8): p. 753-64.

18. Hoemann, C.D., et al., Tissue engineering of cartilage using an injectable and adhesive chitosan-based cell-delivery vehicle. Osteoarthritis Cartilage, 2005. **13**(4): p. 318-29.

19. Yeo, C. and A. Mathur, Autologous bone marrow-derived stem cells for ischemic heart failure: REGENERATE-IHD trial. Regen Med, 2009. **4**(1): p. 119-27.

20. Snowden, J.A., E. Martin-Rendon, and S.M. Watt, Clinical stem cell therapies for severe autoimmune diseases. Transfus Med, 2009. **19**(5): p. 223-34.

21. Nicodemus, G.D. and S.J. Bryant, Cell encapsulation in biodegradable hydrogels for tissue engineering applications. Tissue Eng Part B Rev, 2008. **14**(2): p. 149-65.

22. Spencer, N.J., D.A. Cotanche, and C.M. Klapperich, Peptide- and collagen-based hydrogel substrates for in vitro culture of chick cochleae. Biomaterials, 2008. **29**(8): p. 1028-42.

23. Lu, W.N., et al., Functional improvement of infarcted heart by co-injection of embryonic stem cells with temperature-responsive chitosan hydrogel. Tissue Eng Part A, 2009. **15**(6): p. 1437-47.

24. Wuertz, K., K. Godburn, and J.C. Iatridis, MSC response to pH levels found in degenerating intervertebral discs. Biochem Biophys Res Commun, 2009.**379**(4): p.824-9.

25. Vanderhooft, J.L., et al., Rheological properties of cross-linked hyaluronan-gelatin hydrogels for tissue engineering. Macromol Biosci, 2009. **9**(1): p. 20-8.

26. Boontheekul, T., H.J. Kong, and D.J. Mooney, Controlling alginate gel degradation utilizing partial oxidation and bimodal molecular weight distribution. Biomaterials, 2005. **26**(15): p. 2455-65.

27. Hancock, R.E. and H.G. Sahl, Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol, 2006. **24**(12): p. 1551-7.

28. Chen, J., et al., Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. Stroke, 2001. **32**(4): p. 1005-11.

29. Huang, Y.C., et al., Combined angiogenic and osteogenic factor delivery enhances bone marrow stromal cell-driven bone regeneration. J Bone Miner Res, 2005. **20**(5): p. 848-57.

30. Tateishi-Yuyama, E., et al., Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. Lancet, 2002. **360**(9331): p. 427-35.

31. Kalka, C., et al., Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization. Proc Natl Acad Sci U S A, 2000. **97**(7): p. 3422-7.

32. Gumbiner, B.M., Cell adhesion: the molecular basis of tissue architecture and morphogenesis. Cell, 1996. **84**(3): p. 345-57.

33. Fan, Y.W., et al., Culture of neural cells on silicon wafers with nano-scale surface topograph. J Neurosci Methods, 2002. **120**(1): p. 17-23.

34. United States. Congress. House. Committee on Transportation and Infrastructure., Photovoltaic system for Department of Energy Headquarters building : report (to accompany H.R. 798) (including cost estimate of the Congressional Budget Office), in Report / 110th Congress, 1st session, House of Representatives 110-11. 2007, U.S. G.P.O.: Washington, D.C.

35. Vincent, G.K.a.V., Victoria A, The Next Four Decades, The Older Population in the United States: 2010 to 2050. U.S. Census Bureau, 2010.

36. Weisel, J.W., The mechanical properties of fibrin for basic scientists and clinicians. Biophys Chem, 2004. **112**(2-3): p. 267-76.

Chapter 2: Literature Review

Organ or tissue regeneration by cell therapy is called regenerative medicine. Key goal of regenerative medicine is the management of sustainable biological function by repairing, replacing, and regenerating cells, tissues or organs. This is accomplished by introduction of cells to the damaged tissues or organs combined with therapeutic agents. Most of the research in regenerative medicine centers around finding biocompatible cell friendly systems for delivery, proliferation, and restoration of an existing organ [1]. Degenerative diseases and malfunctioning or damaged organs may be treated with cells that can be autologous, or stemming from the same individual, allogeneic, stemming from the same species, or xenogeneic, stemming from a different species.

Organ transplantation is extensively performed globally and each day 79 people receive organ transplants. In most of the cases organ transplantation is the only viable solution for the treatment of organ failure. The number of organs available for transplantation is much less than the number required, and this discrepancy is turning into one of the most important crises in nearly all countries of the world. Most of the patients on the organ donor waiting list die before a suitable transplant becomes available and currently the ratio of organ donor to receiver is 1: 100,014 [2]. According to United States 'Organ Donation and Implantation Statistics" in May 21. 2014, there are a 122,737 people waiting for lifesaving organ transplants in the U.S [3]. Furthermore, obtaining the organs for transplantation necessitates intricate surgeries to collect the organs from the donor. This organ removal procedure must follow legal and ethical issues, as well as the definition of death and consent.

Globally the direct healthcare costs of organ transplants are about $350 billion and the USA is spending more than $100 billion per year for organ transplant related expenditures and therapies [4]. In the developed countries, the elderly are suffering from organ malfunctions or damage; with 8 million spend on surgical procedures annually to treat those patient [5]. As the older population in the US is growing fast, by 2040, the total number of senior citizens suffering from organ malfunction diseases is expected to jump to 70 million and corresponding health care costs are forecast to be 25% of GDP [6].

Apart from donor shortages and increased expenditures, after organ transplantation patients or organ recipients must have lifetime immunosuppression treatments which have side effects, or complications include infections, and the growth of tumors. Noninvasive surgical procedure for organ transplantation also experiences deficiency of donor tissue and donor site morbidity [7].

2.1 Xenotransplantation and artificial organ as organ donor

The gap between organ donors and recipients is widening and without foreseeable growth in organ donors, it is necessary to reduce the dependency on organ donation and prompt alternative solution such as Xenotransplantation (animal to human), artificial organ (fabricated biomechanical devices/organ), gene therapy, and regenerative medicines. Xenotransplantation has a long history since sixteenth century, when animal blood transfusions and bone transplants to human were performed [8]. Xenotransplantations are still rare because the large immune response and the possible spreading of donor animal viruses to organ recepients [9-11]. Without proper diagnosis, malignancies or infections originated from the donor organ may be transmitted to recipients, triggering severe illness even patient death[12, 13].

The development artificial organs will reduce the dependency on organ donors. Artificial organs are made in laboratory form various types of biomaterials. At present artificial mechanical devices that carry out the function of organs have considerable unwanted side effects and same time reduces the quality of life. However, they sustain life and, with further advances in nanotechnology, stem cells, genetic science, diagnostic, and sensing technologies artificial organs may save millions of critical patience from early death [14].

2.2 Regenerative medicine (RM)

Regenerative medicine is a term that encompasses cell therapy, organ repair, and organ transplantation with various sources. Its goal is to regenerate defective organs, tissues, and cells, by employing cell-based therapies, biomolecular drugs or even medical devices to establish regular function. Cell based therapies promise to offer reliable restorative tools that may be considered for patients at the last stage if their disease [15]. The degenerative diseases targetted by cell based therapies are those involving cells that don't regrow by themselves, such as β-cells, schwann cells, cardiomyocytes and urothelial cells for bladder. A possible solution for these diseases is restoration of damaged cells or tissues by administering healthy donor cells. Regenerative medicine has the potential to cure these illnesses by restoration malfunctioning tissues, provided a good source of cells can be found. Although regenerative medicine is in its infancy state, in recent years significant advances were made in designing novel biomaterials [12], developing new cells delivery mechanism and in characterizing and isolating stem cells as a source of donor cells [16]. Thus regenerative medicine is potentially substituting the organ requirement for transplantation.

2.3 Cell therapy as regenerative medicine

Cell therapy is a subgroup of regenerative medicine, simply defined as the procedure of introducing new cells as therapeutic agents into a damaged tissue aiming to treat disease. It includes the delivery of progenitor cells or stem cells from autologous or allogenieic cell sources into a transplant *in vitro* and *in vivo* [17]. Precursor of modern cellular therapy was pioneered in early 1950 [18], currently achieved milestone progress in cell transplantation from both autologous [19], and allogeneic cell [20] sources. At present biomedical scientists are conducting intensive research in cell-based regenerative therapeutics using non-specialized cell such as autologous and allogeneic cell sources. This therapeutics has been developed to commercialize various cells to improve patient care as well as treating diseases suffering from cell damages. These non-specialized biological cells are very potential to transform into the primary layers of cells from tissues by self-renewal process and provide an infinite source of clinically usable cells for regenerative medicine. Advances in stem cell biology and immunology have significantly ensured the high quality application of cell transplantation as a curative approach.

The omnipotent stem cell is called the mother of cells, because it has the potential to differentiate into specific cell types such as brain, bones, heart, blood, skin, and muscles. Sources of adult stem cells are bone marrow, fat, skeletal muscle, hair follicles, dental pulp, and other, but these are not omnipotent, and some are only giving rise to one specialized cell type. All stem cells have self-renewal capability for long periods, can proliferate extensively and can give rise to specialized cell types. Some studies have examined the effect of stem cells injected into the heart [21] and others looked at their effect on neural diseases like Alzheimer's disease (AD) [22], amyotrophic lateral sclerosis (ALS) [23, 24], Parkinson's disease (PD) [25], Huntington's disease (HD) [26], and spinal cord injury [27]. Successful delivery of stem cells can treat all the

diseases, as they are capable of differentiating into required cell types. In post-delivery stage, these mature cells replace tissues and can produce insulin that is damaged by disease or injury.

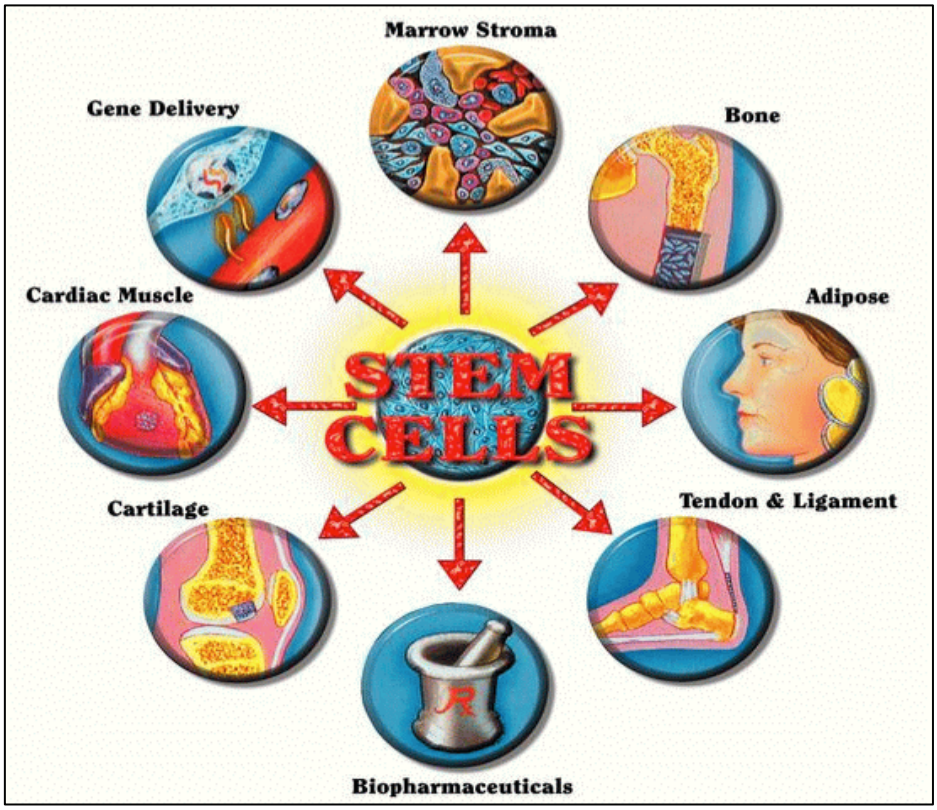

Figure 2.1: Potential of stem cell (Source: National Institutes of Health, Bethesda, MD)

Cell therapy is divided into two modes. In the first mode cells are injected systemically or locally to the site of injury. The delivered cells participate in the healing mechanism by integrating into injured regions and or replacing the damage tissues [28, 29]. In the second mode, delivered cells remain at the injury sites for comparatively small period of time and die within a few days to weeks without further differentiating. Prior to their death cells, they release therapeutic agents such as growth factors; chemokines, and cytokines to aid in the reconstruction

of native tissue [30, 31]. A wide variety of cells types such as neural stem cells [33], myoblasts [34], bone marrow-derived cells [35], and embryonic stem cells [36] have been transplanted as cell therapy. Most of the cells remain in circulation system during post-delivery. Studies have demonstrated that therapeutic action of delivered cells is low, because 75- 90% cells die prior to arrive at the site of injury or damaged area [37].

2.4 Cell delivery methods

Depending on the nature of the injury, type of damage, and its location, various types of cellular constructs can be chosen, each of them having a particular delivery method associated with it. Selection of the delivery method aims at high cell retention, minimal side effects, and high cell survival rates. In addition, the delivered cells must incorporate with the host tissue near the injured region. Two types of cell methods are used in cell therapy: (i) Scaffold based, consisting cells and biomaterials [38], and (ii) biological cell and/or full tissue engineered cell assemblies [39]. Most common are delivery into the diseased tissue directly by intraarticular, intramuscular or intratendinous injection of cells [40]. In some cases, the damage sites necessitated filling or bridging materials in addition to cells, and therefore, cells needed to be delivered together with a matrix [41, 42]. In most cases, this matrix, or scaffolding, serves as a transient support for cells to attach and produce new, organ or tissue-specific extracellular matrix. Scaffold materials must have suitable surface chemistry and the required mechanical strength to promote these cellular activities [43, 44]. A large number of natural and synthetic materials, including some composite materials have been used as scaffolding material described below.

2.4.1 Scaffold based cell delivery

Biocompatible scaffolds serve as cell carrier or vehicles for cell delivery. Engineered scaffolds must supply a microenvironment to support cell/tissue growth during the delivery and transplantation stages. Scaffolds can be solids,, nutrient loaded microspheres or gels. Figure 2.2 shows the different forms of scaffolds used as cell delivery vehicles [45]. As a major structural component of the extracellular matrix, collagen type I, and type II are also responsible for cell attachment, proliferation, and differentiation via cell–matrix interactions [46]. Both these collagens are widely used in cell delivery applications, in particular for tissues of the

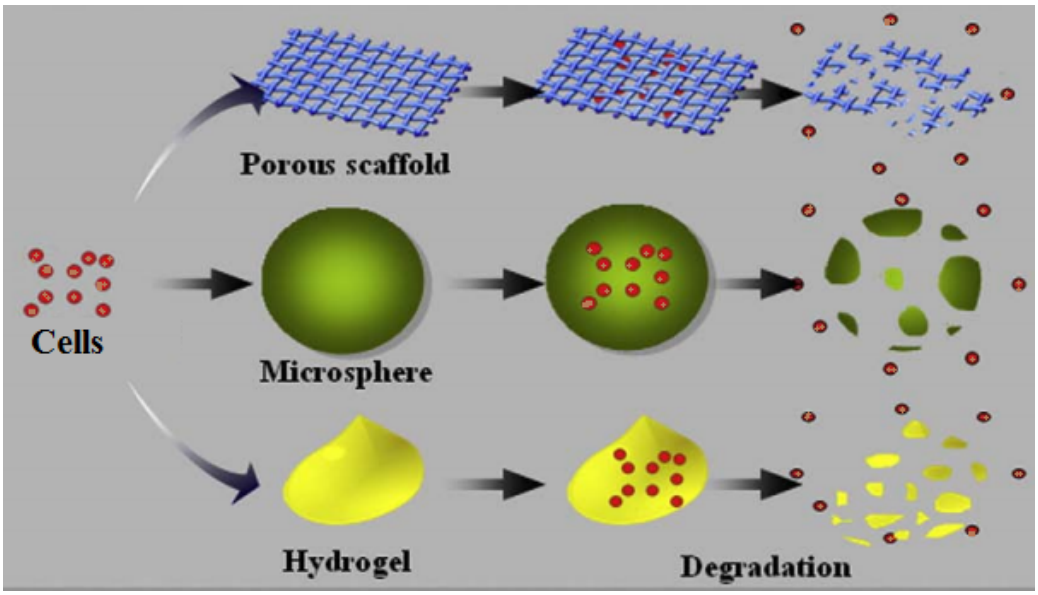

Figure 2.2: Scaffold based cell delivery vehicles [46]

musculoskeletal system [47-50]. They are also used to encapsulate growth factors and cells, which is done in small droplets, microparticles or beads [51-53]. In addition, other natural materials such as fibrin [54], chitosan [55], chondroitin sulfate (CS) [56], and hyaluronan, alginate [57] are used as scaffolding materials. Synthetic biomaterials are also used for targeted

delivery. They offer the advantage that they can be modified chemically to allow for cell specific interactions and they have better mechanical properties compared to natural biomaterials. Most commonly used synthetic biomaterials used as cell delivery vehicles or structural support are polylactic co glycolic acid (PLGA) [58], polycaprolacton (PCL) [59], polyethelene glycol (PEG) [60] and ceramics materials [61].

2.4.2 Scaffold free Delivery System

Scaffold based cell constructs provide initial mechanical and structural support, however, there are still some potential issues regarding with their biocompatibility, biodegradability, and long-term safety. To bypass those risks, scaffold-free cell delivery has been explored as an alternative. Currently three types scaffold free delivery methods are tested in preclinical and clinical trials: (i) ejection of single cells (ii) cell sheet technology and (iii) microtissue technology. These will be briefly reviewed next.

2.4.2.1 Single cells delivery

Scaffold free single cell suspensions are directly delivered to the damage site or tissue via intravenous or intra-arterial injections. The types of cells injected are chosen based on their differentiation potential and the accessibility of the targeted host tissues that requires repair., Direct injection of cells is foremost used for non-vascularized tissues such as intervertebral discs [70] or cartilage [63]. Other target tissues included the pancreas [62], cartilage [63], heart myocardium [64-66], peripheral arteries [67], blood [68], and brain [69]. In these letter cases, the injection can by systemic via intraperitoneal, intravenous or intra-arterial routes, offering the advantage of being less invasive [72]. Systemic cell delivery is best suited for delivery of stem cells such as mesenchymal stem cells [73, 74], or hematopoietic stem cells [74, 76]. In the single

cell delivery systems the injected cells and the host cells interact quickly, however, there is low integration into host tissues and high cellular wash out. Thus very high cell dosages are required for therapeutic success and small dosage results in poor grafting. However, multiple injections using a intracoronary route may have been related to ventricular arrhythmias [77]. Consequently, this delivery method is not widely used and necessitates the investigation of more effective technologies of cell delivery.

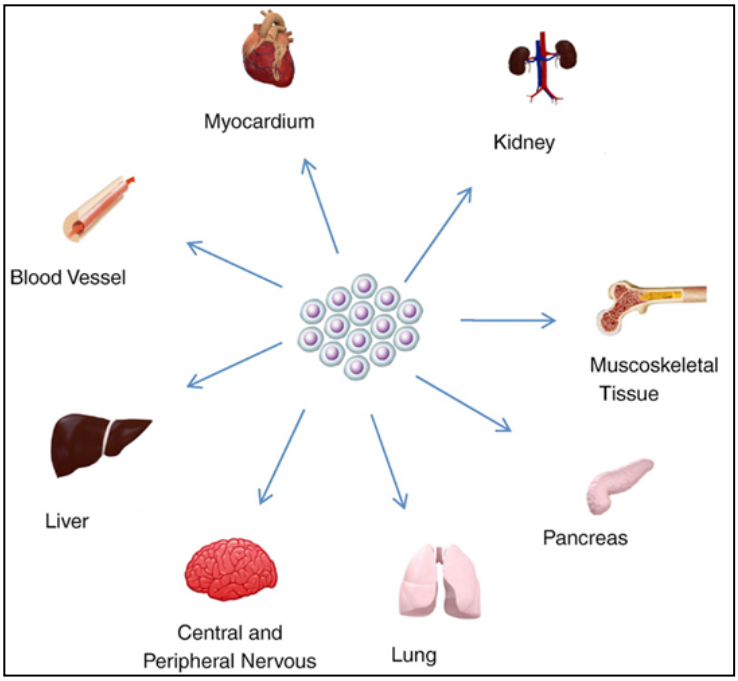

Figure 2.3: Single cell delivery into various tissues [71].

2.4.2.2 Cell sheets delivery

Cell sheet delivery is accomplished first culturing cells until confluence, then detaching the confluent layer from the culturing substrate. The resulting sheet of cells is then transplanted.

Over the last decades in vitro grown cell sheets have been used to regenerate various types of tissues, including corneas [78], full biological small diameter vessels [38], cardiac tissues [16], and whole pancreas [79]. The sources of cells included limbal stem cell, human vascular smooth muscle cells, endothelial cells, beta cells and myocytes. The most common method to collect the cell sheet is to use a thermo reversible material as the cell culturing substrate. When the cell sheet is ready to be collected, the temperature is dropped from 36^{o} to 25^{o}C and the cell sheet floats to the surface of culture dish. Kushida and coworkers developed cell sheet from a temperature-responsive polymer culture surface [80] using human [81] or rabbit limbal cells [78].

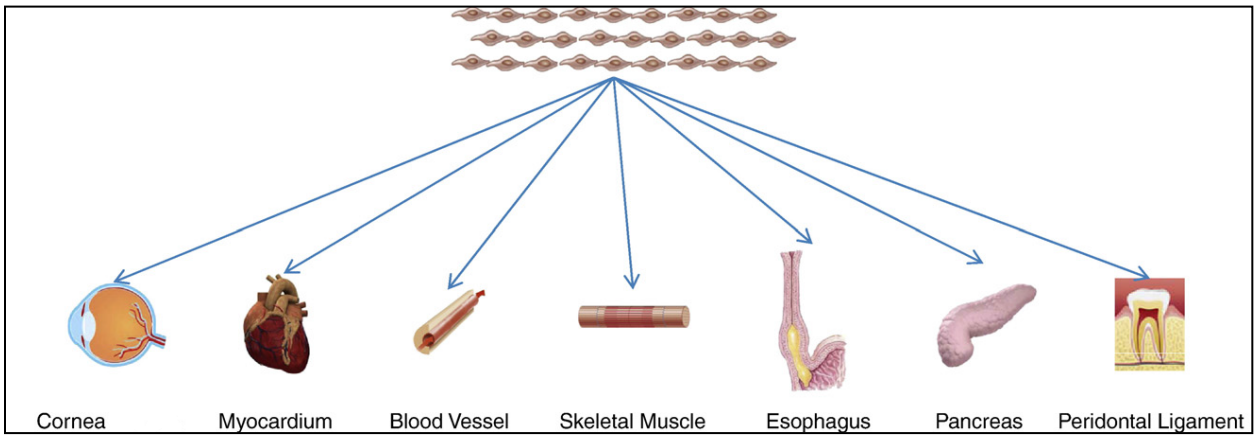

Figure 2.4: Organs for cell sheet delivery to make various bio-processed organs [71].

Cell sheets are also fabricated from magnetization and demagnetization of RGD (Arg-Gly-Asp) coated cell culture surfaces [82]. Laboratory grown cell sheet have been used to generate or repair cardiac [16], cornea [78], skin [83], esophagus [84], and many more. Cell sheets harvested from cell culture substrates are used to produce three dimensional tissues by stacking [16] to standard patches have been made from MSCs [85], fibroblast-endothelial

cocultures [86], neonatal cardiomyocytes [87], skeletal myoblasts [88, 89], or neonatal rat cardiomyocytes endothelial cell cocultures [90]. Cell sheet mediated regenerative approach has huge prospect, however, some limitation need to be addressed such as cell sheets' flimsiness and handling problem throughout surgery [89].

2.4.2.3 Micro-tissues

Micro-tissues are cell clustered normal tissue constructs sized between 100 µm and 500 µm in diameter or thickness generated from individual cells under regulated cell culture conditions. Surgically implantable microtissues can be made from adherent stem cells [91], cell lines [92], and other tissues of interest [93]. Typically, the cells are placed on a non-adherent dish, where the cells form spheres, spheroids, or aggregates (Figure 2.5) [94]. The Injection of microtissues is minimally invasive; furthermore, they offer significant advantages over single cell or cell sheet delivery. Microtissues do not wash out as much as single cell due to relatively larger size. As a result, they adhere faster to extracellular matrix [95] and graft more efficiently to the host tissues.

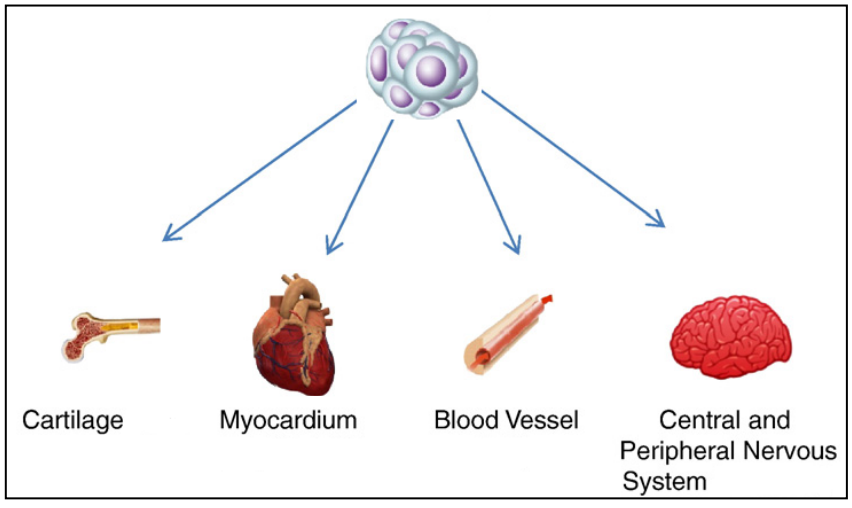

Figure 2.5: Overview of microtissue delivery to repair organ [71].

One of the major concerns in transplantable microtissue, is vascularization, without which the microtissues cannot survive. Microtissues have been shown to generate vascular endothelial growth factor that induces angiogenesis after implantation [96, 97]. Layering of microtissues can also be done. Kelm *et al*. generated living small diameter blood vessels from Human artery-derived fibroblasts and endothelial cells that mimic the native blood vessel morphology. These small diameter grafts offer a huge advantage in comparison to the synthetic grafts used today [95].

2.5 Cell delivery techniques

Regeneration of cells by therapeutic cell delivery advanced the field of regenerative medicine. Currently several types of cell delivery strategy are available; intravenous infusion [98], surgical direct injection [99], catheter based delivery [100], and engineered cell delivery [101]. injection [99], catheter based delivery [100], and engineered cell delivery [101]. The intravenous cell delivery process is noninvasive; however, the efficacy is limited because cells tend to cluster before spreading to the target site. Cell delivery during surgery results in high cell engraftment and viability, but there is potential risk associated with the surgical procedure. Catheter based delivery releases cells near the target sites, but the catheter can cause microembolisms and it can't be used if the artery occluded. Current research focuses on improving techniques for better cell grafting in the target region and improved therapeutic effects of the delivered cells. Table 3.1 shows the advantages and disadvantages of the various cell delivery systems. Using engineering cell delivery the release of the cells can be better controlled and the cells can be directed to the target site by external modulation, potentially improving both grafting and therapeutic effects.

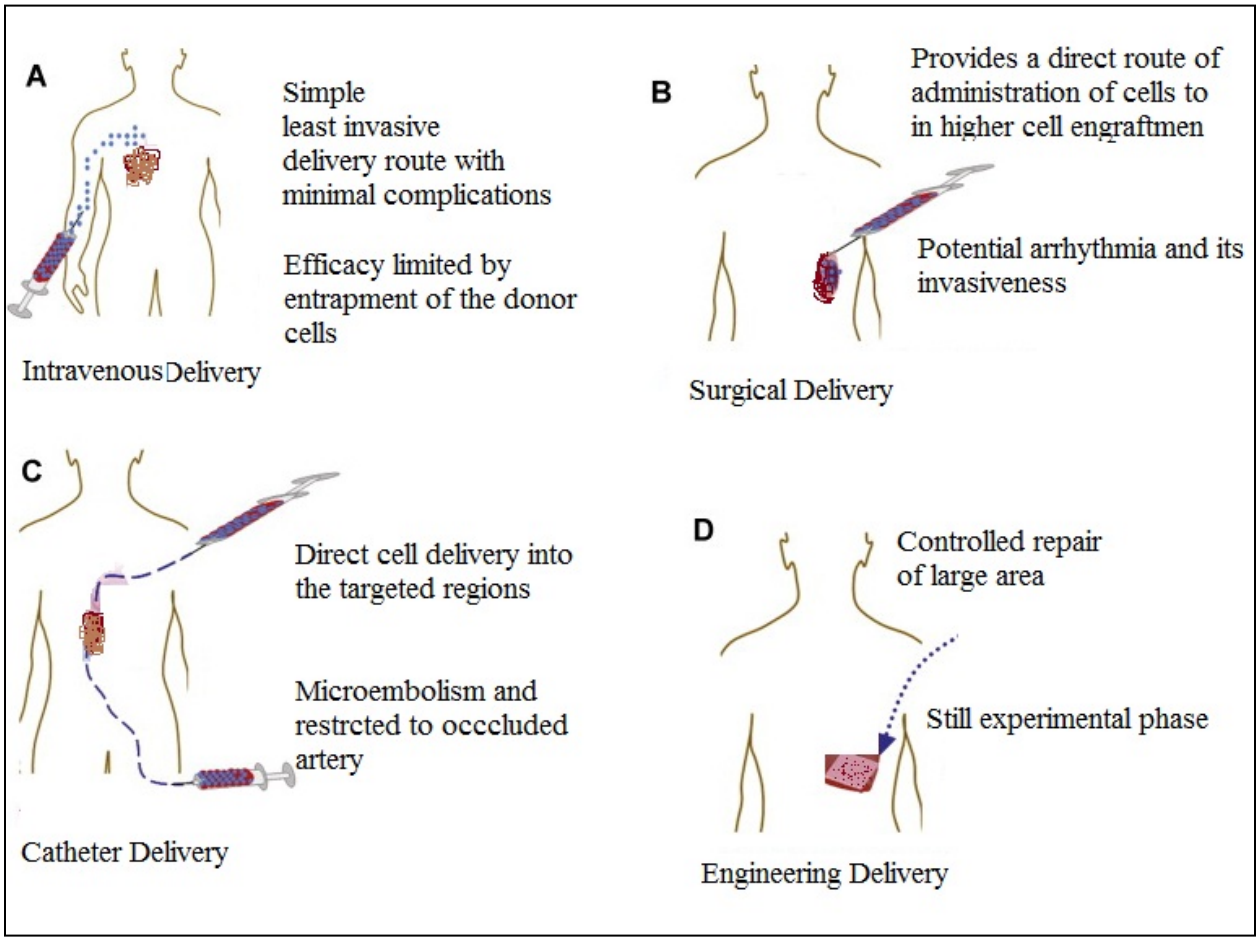

Figure 2.6: Cell delivery strategy for regenerative cell therapy, A [98], B [99], C [100], and D [101].

Table 3.1: Summary of existing cells delivery methods.

Cell delivery method	Advantages	Disadvantages
Surgical delivery method [102]	▪ The most direct cell delivery process. ▪ Precise and accurate approach. ▪ No troubling of surrounding	▪ Noninvasive operation required. ▪ High risks for side effect and mortality. ▪ Reduced cell retention to target side.

	tissue and vasculature.	
Catheter-Based cell Administration [103]	▪ Repeatable as required due to its less invasiveness ▪ Low risk for potential wall perforations.	▪ Requires extensive imaging guidance. ▪ Limited by the scope and development of catheter technology.
Intravenous Injection [104]	▪ Safe and feasible ▪ No arrhythmias ▪ Greater cell retention. Noninvasive delivery route.	▪ Difficult to deliver cells to the right territory. ▪ Induce shear stress to the cell during delivery.
Engineering cell delivery [103]	▪ Great potential for regeneration. ▪ Better cell entrapment. ▪ Huge room available for improvement.	▪ Still in infancy level, leaving more questions than answers. ▪ More inquiry required to validate clinical potential

2.5.1 Cells directing by external modulation

The fundamental strategy of directing injected cells by external modulation is the subject the cell carriers to various physical stimuli which can be controlled externally. This allows more accurate cell delivery. Externally applied stimuli include light, magnetic, thermal, and electrochemical, but this review will be limited to magnetic and thermal stimuli.

2.5.1.1 Cell release controlled by magnetic force

In vitro studies show that magnetic beads that can be used to label cells, also serve as good carriers for delivery of mesenchymal stem cells (MSCs). Magnetically labeled MSCs exhibit better extraction or release rates from the petridish when they are located in an external magnetic field [106]. Kobayashi T. et al [107] investigated the delivery of MSC's labeled with magnetic beads to treat osteochondral defects by intra-articular cell injection. The group used ferumoxides, super paramagnetic iron oxide nanoparticles, that were coated with dextran and poly-L-lysine (PLL). The particles could act as a transfection agents, entering MSCs and also be guided by an externally applied magnetic field.

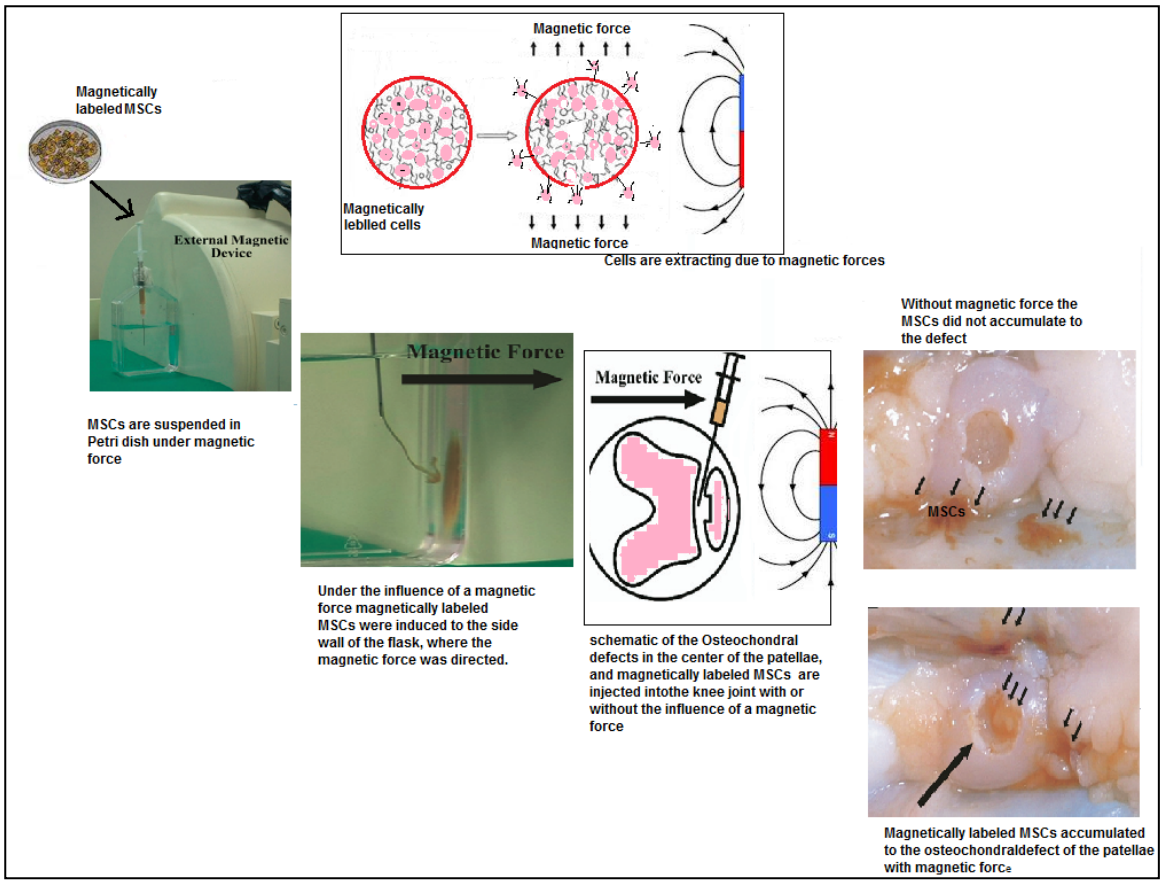

Figure 2.7: Magnetically cell delivery principle and the experimental procedure [107]

into osteochondral defects. This new cell delivery process by magnetic force is very promising for cartilage repair, or osteochondral restoration; however, the authors identified some issues that needed to be properly addressed. The needle or catheter must be nonmagnetic, the lipid bilayer cell membrane may be damaged by inappropriate magnetic force and the, magnetic force might induce the accumulation of all cells in some spots rather than inducing a uniform distribution. In addition, cell viability was not properly addressed in this study.

2.5.1.2 Cell sheet released with temperature modulation

Temperature sensitive cell culturing dishes were used for cell sheet release studies. The key to the innovation is the use of temperature-sensitive materials as cell culture substrates [108]. As one example, Nishida et al. [78] grew corneal epithelial cells on temperature-sensitive petridishes for 2 weeks., Cell sheets were detached from the culturing surface by reducing the temperature to 25°C , collected and implanted cornea of rabbits whose rabbits' conjunctival tissue had been removed. Cells transfer with this procedure was robust, and multilayered cell sheets promoted restoration of the corneal stroma *in vivo* without major surgical operation. Even though the cell sheet procedure is non-invasive, the delivery of the sheets pose a problem, as many potential sites cannot be accessed by a catheter or are as openly accessible as the eye. One potential problem with the technique is the induction of cold stress due to the temperature reduction. Cold stress might interfere with the normal biological function cells or tissues. Furthermore, *in vitro* to *in vivo* handling and implantation of the harvested cell sheets is very risky. Wrinkle or folding of cell sheets or cell damage might lead to scar tissue on the implant. Furthermore, cells cannot grow beyond a monolayer or on the top of each other.

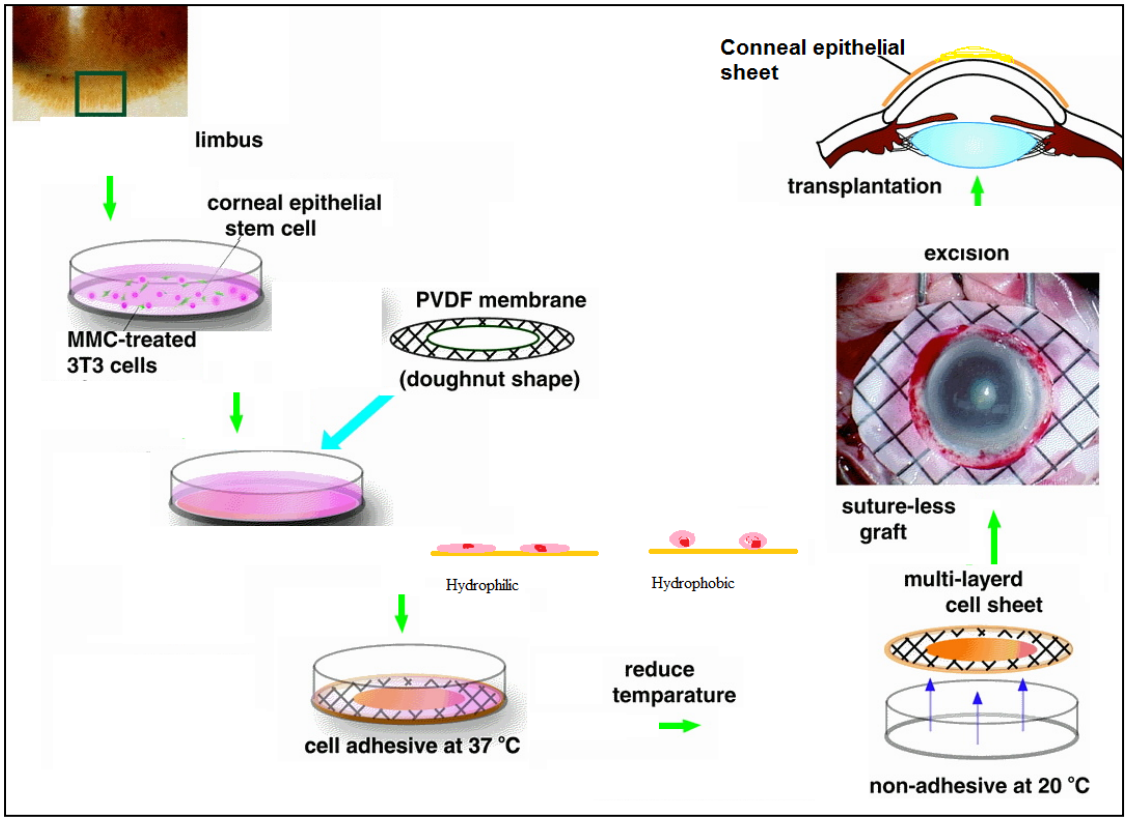

Figure 2.8: Cell delivery as cell sheet with the aid of temperature reduction [78]

The physically modulated approaches described above provide several advantages for cell injection and tissue reconstruction; however, some limitations remain. One of the major problems is the considerable amount of cell loss; only a small fraction of cells are available for the regeneration process at the targeted site. In addition, in injured tissues, the cell supportive structure is already damaged and transferred cells are often not able to attach to the injured architecture. Cell sheet production requires long time and is susceptible to damage or disintegration through handling [39], [89]. Furthermore, the cells can be affected by cold stress and it can potentially damage cell membranes. Therefore, cell delivery by physically modulated approaches often result in random outcomes after implantation.

2.6 Silicon based PV device and its biological interaction

Silicon has seen increasing use as a biomaterial in a wide range of biomedical devices. Some examples are implantable sensors [109], neural electrodes [110], and devices for drug delivery [111], In addition, micro electro mechanical systems are made from crystalline silicon and they have been found to be biocompatible. Apart from biocompatibility, semiconducting properties of silicon are used in photovoltaic (PV) devices. With exposure of light PV generate electricity by the photovoltaic effect creating a voltage difference [112] in between n-type and p-type doped regions; one exhibiting an excess of electrons, the other an electron deficit, respectively referred to as *n*-type doped and *p*-type doped [113] shown in Figure 2.9. By placing metallic contact on the *n* and *p* regions, a diode is obtained. When the junction is illuminated,

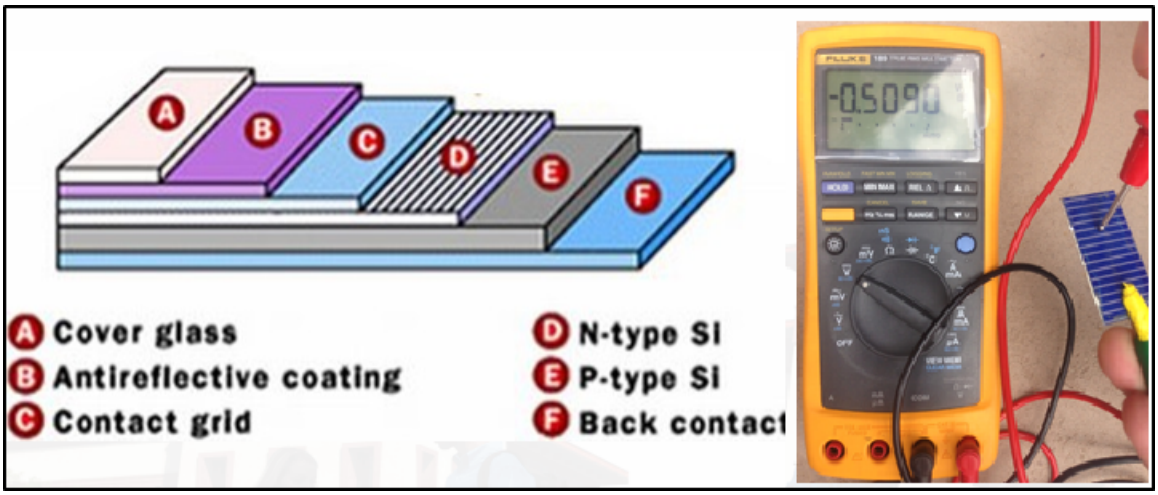

Figure 2.9: Cross section of Silicon based PV devices that generate voltage on the between top and bottom surface.

photons yield their energy to the atoms, each photon causing an electron to move from the valence band to the conduction band, leaving behind a hole, which also allows it to moves

around the material, thus giving rise to an electron-hole pair. Because of the circuit at the cell's terminals, electrons from the *n* region will migrate back to the holes in the *p* region, and it raises the voltage difference by passing electron through circuit [114].

2.7 Electrically excitable cells

Electrically excitable cells are specialized cell in which the cell membrane is polarized and can be depolarized and subsequently repolarized. Cell membranes are made up of lipid bilayers, and ion channels that are located in cell membrane as macromolecular pores.

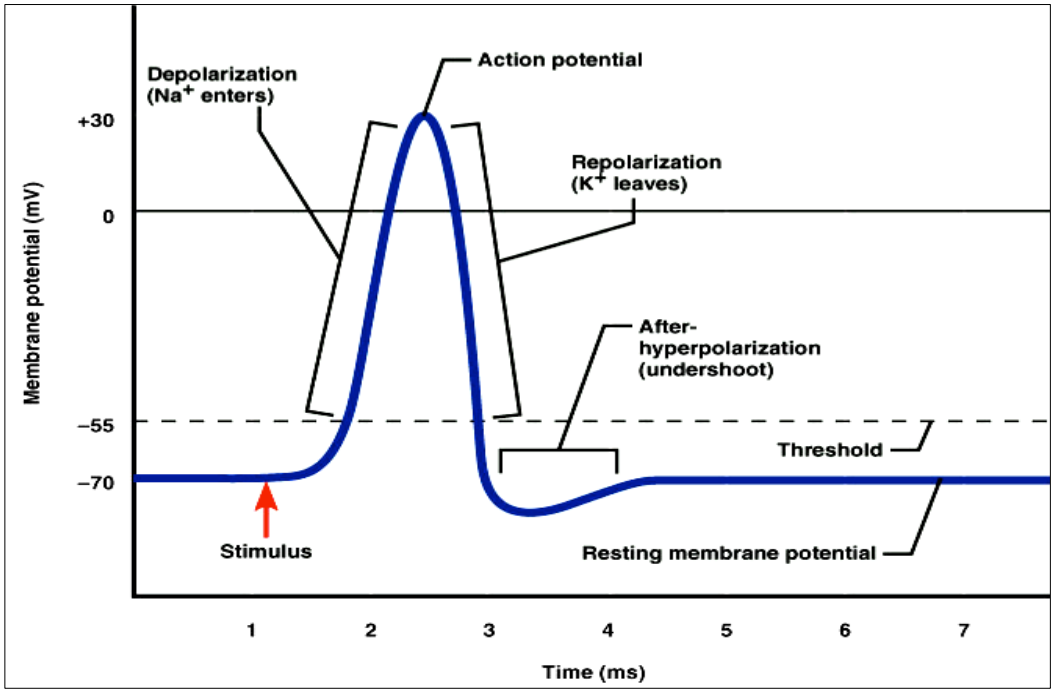

Figure 2.10: Action potential of electrically excitable cells (Source: Pearson Education Inc.)

The ion channels control ion exchange, and thereby the electrical potential of cell membrane [115]. Voltage-gated ion channels are governed by electrical signals to transfer of

specific ions such as Na^+ and K^+ [116]. In the cells, concentration of ions varies with time and depending on location compare to membrane. Na^+ is abundant in outside of cell membrane compare to inside of ell. However, for K^+ the ionic concentration is opposite. Intracellular K^+ concentration higher compare to outside of cell membrane. As result in normal unexcited state cell membrane has an electrical potential of -70mV due to ionic concentration gradient. In excitable state depolari-zation causes an intracellular flow of Na^+ via sodium ion channels make more positive or less negative followed by out ward movement of K^+ ion causes repolarization and hyperpolarization making more negative membrane potential [117]. Action potential in cells is defined as a transient phenomena in which electrical potential or voltage of a cell membrane sequentially rises and falls in a rapid manner [118]. Action potentials take place in muscle cells [119], fertilized eggs [120], neurons, [121], and endocrine cells [122].

References

1. Yamamoto, M. and Y. Tabata, Tissue engineering by modulated gene delivery. Advanced drug delivery reviews, 2006. **58**(4): p. 535-54.
2. Bureau, U.S.C., National health expenditures by type. Statistical Abstract of the United States, 1990-2000: p. 92.
3. Network, U.S.D.o.H.H.S.O.P.a.T., ORGAN DONATION AND TRANSPLANTATION STATISTICS. 2014.
4. Langer, R. and J.P. Vacanti, Tissue engineering. Science, 1993. **260**(5110): p. 920-6.
5. Niklason, L.E. and R. Langer, Prospects for organ and tissue replacement. JAMA, 2001. **285**(5): p. 573-6.
6. Sutton, M.G. and N. Sharpe, Left ventricular remodeling after myocardial infarction: pathophysiology and therapy. Circulation, 2000. **101**(25): p. 2981-8.

7. Fuchs, J.R., B.A. Nasseri, and J.P. Vacanti, Tissue engineering: a 21st century solution to surgical reconstruction. Ann Thorac Surg, 2001. **72**(2): p. 577-91.

8. Deschamps, J.Y., et al., History of xenotransplantation. Xenotransplantation, 2005. **12**(2): p. 91-109.

9. Mueller, N.J., et al., Activation of cytomegalovirus in pig-to-primate organ xenotransplantation. J Virol, 2002. **76**(10): p. 4734-40.

10. Patience, C., Y. Takeuchi, and R.A. Weiss, Infection of human cells by an endogenous retrovirus of pigs. Nat Med, 1997. **3**(3): p. 282-6.

11. Stock, U.A. and J.P. Vacanti, Tissue engineering: current state and prospects. Annu Rev Med, 2001. **52**: p. 443-51.

12. Srinivasan, A., et al., Transmission of rabies virus from an organ donor to four transplant recipients. N Engl J Med, 2005. **352**(11): p. 1103-11.

13. Buell, J.F., et al., Donor transmitted malignancies. Ann Transplant, 2004. **9**(1): p. 53-6.

14. Polak, J.M., L.L. Hench, and P. Kemp, Future strategies for tissue and organ replacement. 2002, LondonRiver Edge, NJ: Imperial College Press ;Distributed by World Scientific Pub. xix, 427 p.

15. Slavin, S., et al., Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. Blood, 1998. **91**(3): p. 756-63.

16. Lee, S.O., et al., New therapy targeting differential androgen receptor signaling in prostate cancer stem/progenitor vs. non-stem/progenitor cells. J Mol Cell Biol, 2013. **5**(1): p. 14-26.

17. Riazi, A.M., S.Y. Kwon, and W.L. Stanford, Stem cell sources for regenerative medicine. Methods Mol Biol, 2009. **482**: p. 55-90.

18. Kemeny, D.M. and P.A. MacAry, Immunology: making magic bullets. BMJ, 2007. **334 Suppl 1**: p. s13.

19. Yamanaka, J.B.G.a.S., The Nobel Prize in Physiology or Medicine 2012. Nobelprize.org, 2012.

20. Starzl, T.E., History of clinical transplantation. World J Surg, 2000. **24**(7): p. 759-82.

21. Breitbart, A.S., et al., Gene-enhanced tissue engineering: applications for bone healing using cultured periosteal cells transduced retrovirally with the BMP-7 gene. Ann Plast Surg, 1999. **42**(5): p. 488-95.

22. 29. Wang, J.M., et al., Allopregnanolone reverses neurogenic and cognitive deficits in mouse model of Alzheimer's disease. Proc Natl Acad Sci U S A, 2010. **107**(14): p. 6498-503.

23. Mazzini, L., et al., Mesenchymal stem cell transplantation in amyotrophic lateral sclerosis: A Phase I clinical trial. Exp Neurol, 2010. **223**(1): p. 229-37.

24. Mazzini, L., et al., Stem cell therapy in amyotrophic lateral sclerosis: a methodological approach in humans. Amyotroph Lateral Scler Other Motor Neuron Disord, 2003. **4**(3): p. 158-61.

25. Wernig, M., et al., Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. Proc Natl Acad Sci U S A, 2008. **105**(15): p. 5856-61.

26. Bloch, J., et al., Neuroprotective gene therapy for Huntington's disease, using polymer-encapsulated cells engineered to secrete human ciliary neurotrophic factor: results of a phase I study. Hum Gene Ther, 2004. **15**(10): p. 968-75.

27. Yoon, S.H., et al., Complete spinal cord injury treatment using autologous bone marrow cell transplantation and bone marrow stimulation with granulocyte macrophage-colony stimulating factor: Phase I/II clinical trial. Stem Cells, 2007. **25**(8): p. 2066-73.

28. Jackson, K.A., et al., Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest, 2001. **107**(11): p. 1395-402.

29. Kawada, H., et al., Nonhematopoietic mesenchymal stem cells can be mobilized and differentiate into cardiomyocytes after myocardial infarction. Blood, 2004. **104**(12): p. 3581-7.

30. Deuse, T., et al., Hepatocyte growth factor or vascular endothelial growth factor gene transfer maximizes mesenchymal stem cell-based myocardial salvage after acute myocardial infarction. Circulation, 2009. **120**(11 Suppl): p. S247-54.

31. Kelly, M.L., et al., TNF receptor 2, not TNF receptor 1, enhances mesenchymal stem cell-mediated cardiac protection following acute ischemia. Shock, 2010. **33**(6): p. 602-7.

32. Nugent, H.M., et al., Delivery site of perivascular endothelial cell matrices determines control of stenosis in a porcine femoral stent model. J Vasc Interv Radiol, 2009. **20**(12): p. 1617-24.

33. Lu, P., et al., Neural stem cells constitutively secrete neurotrophic factors and promote extensive host axonal growth after spinal cord injury. Exp Neurol, 2003. **181**(2): p. 115-29.

34. Skuk, D., et al., Ischemic central necrosis in pockets of transplanted myoblasts in nonhuman primates: implications for cell-transplantation strategies. Transplantation, 2007. **84**(10): p. 1307-15.

35. Hofmann, M., et al., Monitoring of bone marrow cell homing into the infarcted human myocardium. Circulation, 2005. **111**(17): p. 2198-202.

36. Laflamme, M.A., et al., Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. Nat Biotechnol, 2007. **25**(9): p. 1015-24.

37. Mooney, D.J. and H. Vandenburgh, Cell delivery mechanisms for tissue repair. Cell Stem Cell, 2008. **2**(3): p. 205-13.

38. Lutolf, M.P. and J.A. Hubbell, Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. Nat Biotechnol, 2005. **23**(1): p. 47-55.

39. L'Heureux, N., et al., A completely biological tissue-engineered human blood vessel. FASEB J, 1998. **12**(1): p. 47-56.

40. Noth, U., A.F. Steinert, and R.S. Tuan, Technology insight: adult mesenchymal stem cells for osteoarthritis therapy. Nat Clin Pract Rheumatol, 2008. **4**(7): p. 371-80.

41. Marcacci, M., et al., Stem cells associated with macroporous bioceramics for long bone repair: 6- to 7-year outcome of a pilot clinical study. Tissue Eng, 2007. **13**(5): p. 947-55.

42. Brittberg, M., Autologous chondrocyte implantation--technique and long-term follow-up. Injury, 2008. **39 Suppl 1**: p. S40-9.

43. Kaul, G., et al., Failed cartilage repair for early osteoarthritis defects: a biochemical, histological and immunohistochemical analysis of the repair tissue after treatment with

marrow-stimulation techniques. Knee Surg Sports Traumatol Arthrosc, 2012. **20**(11): p. 2315-24.

44. Chen, F.H., K.T. Rousche, and R.S. Tuan, Technology Insight: adult stem cells in cartilage regeneration and tissue engineering. Nat Clin Pract Rheumatol, 2006. **2**(7): p. 373-82.

45. Huang, S. and X. Fu, Naturally derived materials-based cell and drug delivery systems in skin regeneration. J Control Release, 2010. **142**(2): p. 149-59.

46. Friess, W., Collagen--biomaterial for drug delivery. Eur J Pharm Biopharm, 1998. **45**(2): p. 113-36.

47. Dawson, J.I. and R.O. Oreffo, Bridging the regeneration gap: stem cells, biomaterials and clinical translation in bone tissue engineering. Arch Biochem Biophys, 2008. **473**(2): p. 124-31.

48. Dawson, E., et al., Biomaterials for stem cell differentiation. Advanced drug delivery reviews, 2008. **60**(2): p. 215-28.

49. Khan, Y., et al., Tissue engineering of bone: material and matrix considerations. J Bone Joint Surg Am, 2008. **90 Suppl 1**: p. 36-42.

50. Munirah, S., et al., The use of fibrin and poly(lactic-co-glycolic acid) hybrid scaffold for articular cartilage tissue engineering: an in vivo analysis. Eur Cell Mater, 2008. **15**: p. 41-52.

51. Gaissmaier, C., et al., Effect of human platelet supernatant on proliferation and matrix synthesis of human articular chondrocytes in monolayer and three-dimensional alginate cultures. Biomaterials, 2005. **26**(14): p. 1953-60.

52. Akeda, K., et al., Platelet-rich plasma stimulates porcine articular chondrocyte proliferation and matrix biosynthesis. Osteoarthritis Cartilage, 2006. **14**(12): p. 1272-80.

53. Steinert, A., et al., Chondrogenic differentiation of mesenchymal progenitor cells encapsulated in ultrahigh-viscosity alginate. J Orthop Res, 2003. **21**(6): p. 1090-7.

54. Scotti, C., et al., Healing of meniscal tissue by cellular fibrin glue: an in vivo study. Knee Surg Sports Traumatol Arthrosc, 2009. **17**(6): p. 645-51.

55. Di Martino, A., M. Sittinger, and M.V. Risbud, Chitosan: a versatile biopolymer for orthopaedic tissue-engineering. Biomaterials, 2005. **26**(30): p. 5983-90.

56. Ferdous, Z. and K.J. Grande-Allen, Utility and control of proteoglycans in tissue engineering. Tissue Eng, 2007. **13**(8): p. 1893-904.

57. George, M. and T.E. Abraham, Polyionic hydrocolloids for the intestinal delivery of protein drugs: alginate and chitosan--a review. J Control Release, 2006. **114**(1): p. 1-14.

58. Li, W.J., Y.J. Jiang, and R.S. Tuan, Cell-nanofiber-based cartilage tissue engineering using improved cell seeding, growth factor, and bioreactor technologies. Tissue Eng Part A, 2008. **14**(5): p. 639-48.

59. Goyenvalle, E., et al., Osteointegration of femoral stem prostheses with a bilayered calcium phosphate coating. Biomaterials, 2006. **27**(7): p. 1119-28.

60. Goyenvalle, E., et al., Bilayered calcium phosphate coating to promote osseointegration of a femoral stem prosthesis. J Mater Sci Mater Med, 2003. **14**(3): p. 219-27.

61. Thomas, C.E., A. Ehrhardt, and M.A. Kay, Progress and problems with the use of viral vectors for gene therapy. Nat Rev Genet, 2003. **4**(5): p. 346-58.

62. Sapir, T., et al., Cell-replacement therapy for diabetes: Generating functional insulin-producing tissue from adult human liver cells. Proc Natl Acad Sci U S A, 2005. **102**(22): p. 7964-9.

63. Brittberg, M., et al., Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med, 1994. **331**(14): p. 889-95.

64. Arnesen, H., et al., Cell therapy in myocardial infarction. Lancet, 2007. **369**(9580): p. 2142-3.

65. Couzin, J. and G. Vogel, Cell therapy. Renovating the heart. Science, 2004. **304**(5668): p. 192-4.

66. Orlic, D., et al., Transplanted adult bone marrow cells repair myocardial infarcts in mice. Ann N Y Acad Sci, 2001. **938**: p. 221-9; discussion 229-30.

67. Al Mheid, I. and A.A. Quyyumi, Cell therapy in peripheral arterial disease. Angiology, 2008. **59**(6): p. 705-16.

68. Antoine, C., et al., Long-term survival and transplantation of haemopoietic stem cells for immunodeficiencies: report of the European experience 1968-99. Lancet, 2003. **361**(9357): p. 553-60.

69. Kim, S.U. and J. de Vellis, Stem cell-based cell therapy in neurological diseases: a review. J Neurosci Res, 2009. **87**(10): p. 2183-200.

70. Sakai, D., Future perspectives of cell-based therapy for intervertebral disc disease. Eur Spine J, 2008. **17 Suppl 4**: p. 452-8.

71. Kelm, J.M. and M. Fussenegger, Scaffold-free cell delivery for use in regenerative medicine. Advanced drug delivery reviews, 2010. **62**(7-8): p. 753-64.

72. Bartunek, J., et al., Delivery of biologics in cardiovascular regenerative medicine. Clin Pharmacol Ther, 2009. **85**(5): p. 548-52.

73. Karp, J.M. and G.S. Leng Teo, Mesenchymal stem cell homing: the devil is in the details. Cell Stem Cell, 2009. **4**(3): p. 206-16.

74. Zhang, M., et al., SDF-1 expression by mesenchymal stem cells results in trophic support of cardiac myocytes after myocardial infarction. FASEB J, 2007. **21**(12): p. 3197-207.

75. Bensinger, W.I., Role of autologous and allogeneic stem cell transplantation in myeloma. Leukemia, 2009. **23**(3): p. 442-8.

76. Murry, C.E., et al., Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature, 2004. **428**(6983): p. 664-8.

77. Rutanen, J., et al., Adenoviral catheter-mediated intramyocardial gene transfer using the mature form of vascular endothelial growth factor-D induces transmural angiogenesis in porcine heart. Circulation, 2004. **109**(8): p. 1029-35.

78. Nishida, K., et al., Functional bioengineered corneal epithelial sheet grafts from corneal stem cells expanded ex vivo on a temperature-responsive cell culture surface. Transplantation, 2004. **77**(3): p. 379-85.

79. White, S.A., J.A. Shaw, and D.E. Sutherland, Pancreas transplantation. Lancet, 2009. **373**(9677): p. 1808-17.

80. Yang, J., et al., Cell sheet engineering: recreating tissues without biodegradable scaffolds. Biomaterials, 2005. **26**(33): p. 6415-22.

81. Sumide, T., et al., Functional human corneal endothelial cell sheets harvested from temperature-responsive culture surfaces. FASEB J, 2006. **20**(2): p. 392-4.

82. O. Guillaume-Gentil, Y.A., M. Schuler, C. Tang, M. Textor, M. Yamato, Polyelectrolyte coatings with a potential for electronic control and cell sheet engineering. Advanced Materials, 2008. **20**: p. 560-565.

83. Yamato, M., et al., Thermo-responsive culture dishes allow the intact harvest of multilayered keratinocyte sheets without dispase by reducing temperature. Tissue Eng, 2001. **7**(4): p. 473-80.

84. Ohki, T., et al., Treatment of oesophageal ulcerations using endoscopic transplantation of tissue-engineered autologous oral mucosal epithelial cell sheets in a canine model. Gut, 2006. **55**(12): p. 1704-10.

85. Miyahara, Y., et al., Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. Nat Med, 2006. **12**(4): p. 459-65.

86. Kobayashi, H., et al., Fibroblast sheets co-cultured with endothelial progenitor cells improve cardiac function of infarcted hearts. J Artif Organs, 2008. **11**(3): p. 141-7.

87. Shimizu, T., et al., Long-term survival and growth of pulsatile myocardial tissue grafts engineered by the layering of cardiomyocyte sheets. Tissue Eng, 2006. **12**(3): p. 499-507.

88. Hoashi, T., et al., Skeletal myoblast sheet transplantation improves the diastolic function of a pressure-overloaded right heart. J Thorac Cardiovasc Surg, 2009. **138**(2): p. 460-7.

89. Hamdi, H., et al., Cell delivery: intramyocardial injections or epicardial deposition? A head-to-head comparison. Ann Thorac Surg, 2009. **87**(4): p. 1196-203.

90. Sekine, H., et al., Endothelial cell coculture within tissue-engineered cardiomyocyte sheets enhances neovascularization and improves cardiac function of ischemic hearts. Circulation, 2008. **118**(14 Suppl): p. S145-52.

91. Keller, G.M., In vitro differentiation of embryonic stem cells. Curr Opin Cell Biol, 1995. **7**(6): p. 862-9.

92. Friedrich, J., et al., Spheroid-based drug screen: considerations and practical approach. Nat Protoc, 2009. **4**(3): p. 309-24.

93. Sanchez-Bustamante, C.D., et al., Modulation of cardiomyocyte electrical properties using regulated bone morphogenetic protein-2 expression. Tissue Eng Part A, 2008. **14**(12): p. 1969-88.

94. Desoize, B. and J. Jardillier, Multicellular resistance: a paradigm for clinical resistance? Crit Rev Oncol Hematol, 2000. **36**(2-3): p. 193-207.

95. Kelm, J.M., et al., A novel concept for scaffold-free vessel tissue engineering: self-assembly of microtissue building blocks. J Biotechnol, 2010. **148**(1): p. 46-55.

96. Kelm, J.M., et al., VEGF profiling and angiogenesis in human microtissues. J Biotechnol, 2005. **118**(2): p. 213-29.

97. Kelm, J.M., et al., Design of custom-shaped vascularized tissues using microtissue spheroids as minimal building units. Tissue Eng, 2006. **12**(8): p. 2151-60.

98. Fischer, U.M., et al., Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary first-pass effect. Stem Cells Dev, 2009. **18**(5): p. 683-92.

99. Amado, L.C., et al., Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. Proc Natl Acad Sci U S A, 2005. **102**(32): p. 11474-9.

100. Smits, P.C., et al., Catheter-based intramyocardial injection of autologous skeletal myoblasts as a primary treatment of ischemic heart failure: clinical experience with six-month follow-up. J Am Coll Cardiol, 2003. **42**(12): p. 2063-9.

101. Suuronen, E.J., et al., Tissue-engineered injectable collagen-based matrices for improved cell delivery and vascularization of ischemic tissue using CD133+ progenitors expanded from the peripheral blood. Circulation, 2006. **114**(1 Suppl): p. I138-44.

102. Hagege, A.A., et al., Skeletal myoblast transplantation in ischemic heart failure: long-term follow-up of the first phase I cohort of patients. Circulation, 2006. **114**(1 Suppl): p. I108-13.

103. Klein, R.M., et al., [Inflammation of the myocardium as an arrhythmia trigger]. Z Kardiol, 2000. **89 Suppl 3**: p. 24-35.

104. Halkos, M.E., et al., Intravenous infusion of mesenchymal stem cells enhances regional perfusion and improves ventricular function in a porcine model of myocardial infarction. Basic Res Cardiol, 2008. **103**(6): p. 525-36.

105. Bel, A., et al., Composite cell sheets: a further step toward safe and effective myocardial regeneration by cardiac progenitors derived from embryonic stem cells. Circulation, 2010. **122**(11 Suppl): p. S118-23.

106. Kost, J., J. Wolfrum, and R. Langer, Magnetically enhanced insulin release in diabetic rats. J Biomed Mater Res, 1987. **21**(12): p. 1367-73.

107. Kobayashi, T., et al., A novel cell delivery system using magnetically labeled mesenchymal stem cells and an external magnetic device for clinical cartilage repair. Arthroscopy, 2008. **24**(1): p. 69-76.

108. Fournier, E., et al., Biocompatibility of implantable synthetic polymeric drug carriers: focus on brain biocompatibility. Biomaterials, 2003. **24**(19): p. 3311-31.

109. Ferrara, L.A., A. J. Fleischman, D. Togawa, T. W. Bauer, E. C. Benzel, and S. Roy, An in vivo biocompatibility assessment of MEMS materials for spinal fusion monitoring. Microdevices, 2003. **5**: p. 297-302.

110. Martinez Santiesteban, F.M., Magnetic Resonance Imaging Study of Silicon Microelectrodes. 2006. 1 v.

111. Desai, T.A., D. Hansford, and M. Ferrari, Characterization of micromachined silicon membranes for immunoisolation and bioseparation applications. J. Membr. Sci, 1999(159): p. 221.

112. Califano, F.P., Current Utilization of Photovoltaic Effect. Chimica & L Industria, 1975.**57**(4): p. 293- 293.

113. Chen, L.J., et al., Photovoltaic effect in a periodically poled lithium niobate Solc-typewavelength filter. Applied Physics Letters, 2006. **88**(12)

114. Ning-Ping Huang, R.M., Janos Voros, Marcus Textor, Rolf Hofer, Antonella Rossi, Donald L. Elbert,Jeffrey A. Hubbell, and Nicholas D. Spence, Poly(l-lysine)-g-poly(ethylene glycol) Layers on Meta Oxide Surfaces: Surface-Analytical Characterization and Resistance to Serum and Fibrinogen Adsorption. American Chemical Society, 2001.

115. Elbert, T., et al., Chaos and physiology: deterministic chaos in excitable cell assemblies. Physiol Rev, 1994. **74**(1): p. 1-47.

116. Barron, J.A., et al., Biological laser printing of genetically modified Escherichia coli for biosensor applications. Biosens Bioelectron, 2004. **20**(2): p. 246-52.

117. Barron, J.A.e.a., Application of laser printing to mammalian cells. Thin Solid Films, 2004: p. 383-387.

118. Barnett, M.W. and P.M. Larkman, The action potential. Pract Neurol, 2007. **7**(3):p.192-7.

119. Jia-Qiang He, Y.M., Youngsook Lee, James A. Thomson, Timothy J. Kamp, Human Embryonic Stem Cells Develop Into Multiple Types of Cardiac Myocytes. Cellular Biology, 2003. **93**: p. 30-39.

120. Sontheimer, H., Voltage-dependent ion channels in glial cells. Glia, 1994. **11**(2): p. 156-72.

121. Ballaro, B.a.R., P.G., Rotating spiral waves in fertilized ascidian eggs.Rivista Di Biologia-Biology Forum 2002. **95**(1): p. 101-112.

122. Gu, Y., et al., High-resolution scanning patch-clamp: new insights into cell function. FASEB J, 2002. **16**(7): p. 748-50.

Chapter 3: Hypothesis and Objective

3.1 Hypothesis

It is hypothesized that light responsive biocompatible photovoltaic devices can be used as substrates for culturing of biological cells. Upon successful culturing of living cells, light stimulation can be used to release cells by electrical charge repulsion.

We will explore whether the biological cells are able to be cultured on the PV devices or not. And if we are successful in culturing the cells, we will test if they can be efficiently removed from the culturing surface upon light exposure or photon stimulation. To proof the concept, we will first conduct biocompatibility tests with a range of nontoxic commercially accessible photovoltaic devices. In these tests, we will determine if the cells can attach to the surfaces of the PV devices.

We will then select the most biocompatible devices and study whether; C2C12 cells can be grown on the devices. After attaining semi-confluence or confluence level, we will induce electrical charges on the device surface by exposing the device to light. Any released cells will be analyzed as shown in Figure 3.1. The fundamental goal of this investigation is to develop a controlled cell release device.

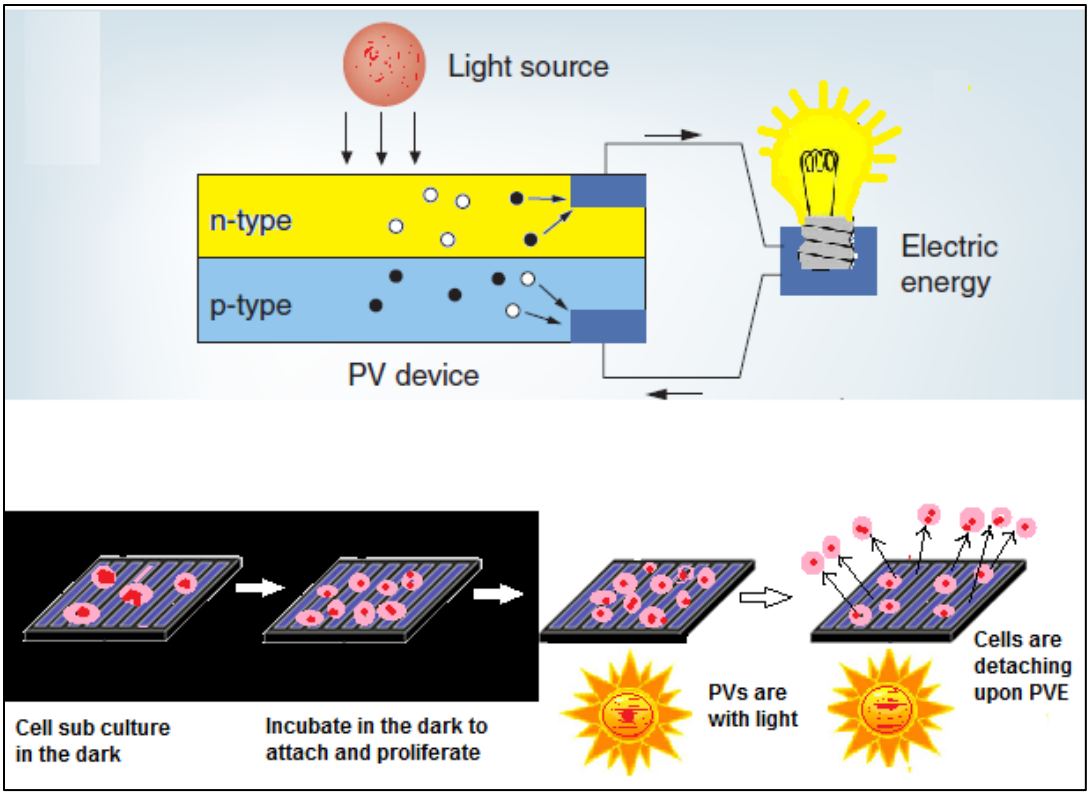

Figure 3.1: PV effect and cell culturing on PV devices

3.2 Objective

a. **Assessing biocompatible PV devices**: To assess the biocompatibility, various PV devices made of nontoxic material were evaluated. C2C12 cells were cultured with small pieces of PV devices and microscopically observed for 2 weeks to detect any harsh effect on cells and cells normal growth profile.

b. Cell culturing on photovoltaic devices: Upon successful assessing of PV devices, C2C12 cells were cultured on PV substrate to observe cells growth profile, cell viability, live/dead assay and total cells count up to confluence level

c. **Release of attached cells using light source:** Cells cultured on PV were released or detached with light sources at confluent cell cultured. Cells count of released cells was compared with total number of attached cells, cells in dark group (non exposure of light) and cells released by enzymatic process by trypsin.

d. **Progressive release of proliferated cells from PV devices and increases the stability phase of cell growth curve:** Growth profile, quantitative and qualitative analysis of remaining cells attached to PV devices after periodically light exposure were evaluated to increase stability phase (without death phase) for successive cell release by nonenzymatic process.

Chapter 4: Materials and Method

In this section, the protocols used to accomplish our goal are described. Preparation of silicon devices as cells substrates, and biological cell seeding on these devices are detailed. The cell release procedure from the culturing devices using light as photo simulation as well as histological and morphological analysis also discussed.

4.1 Materials

4.1.1 Photo voltaic cells

For the experiment we used Photovoltaic cells obtained from RadioShack® (Fort Worth, TX). Monocrystalline silicon based porous PV are made of single crystal structured that shows maximum open circuit surface voltage 0.55VDC in full sunlight (100 Klux), and maximum current 0.25 to 0.275 amps. However, the surface voltage is variable with various light intensities

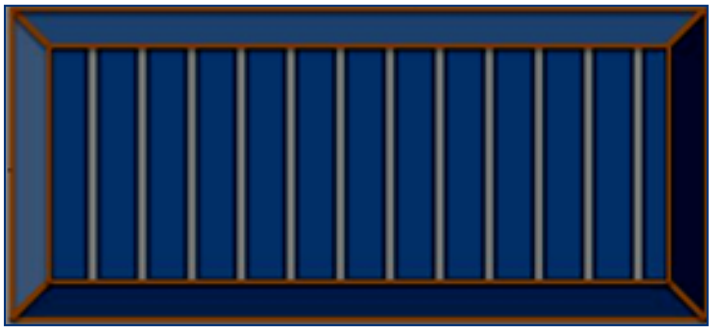

Figure 4: Silicon based PV cells

4.1.2 A boundary wall surrounding the PV cells was made with glue to protect the culturing medium and cell solution from leaking out of the culturing surface. The small round glue sticks was manufactured by Ad tech ™ (Adhesive Technologies Inc., Hampton, NH). The

clear easy bonding adhesive sticks were made from non-toxic material and their melting point is 92°C. Acrylic polymer based adhesive was applied with glue gun to yield a wall of 2 mm thick and 3 mm in height. The curing process of glues was 2 minutes.

Figure 5: Ad tech glue sticks (Adhesive Technologies Inc.)

4.1.3 C2C12 Cell

Anchorage dependent myoblasts C2C12 mammalian were obtained from American Type Culture Collection, ATCC (CRL-1772) grown in Dulbecco's Modified Eagle's Medium (DMEM) augmented with 1% antibiotics, 2mM glutamine, and 10% fetal bovine serum; the pH was adjusted to7.5. The cell line was incubated in an incubator, maintaining 5% CO_2 atmosphere at 37°C, and 100% humidity. Two of the key characteristics of C2C12 cells are that they are electrically excitable and capable of differentiating in culturing environment.

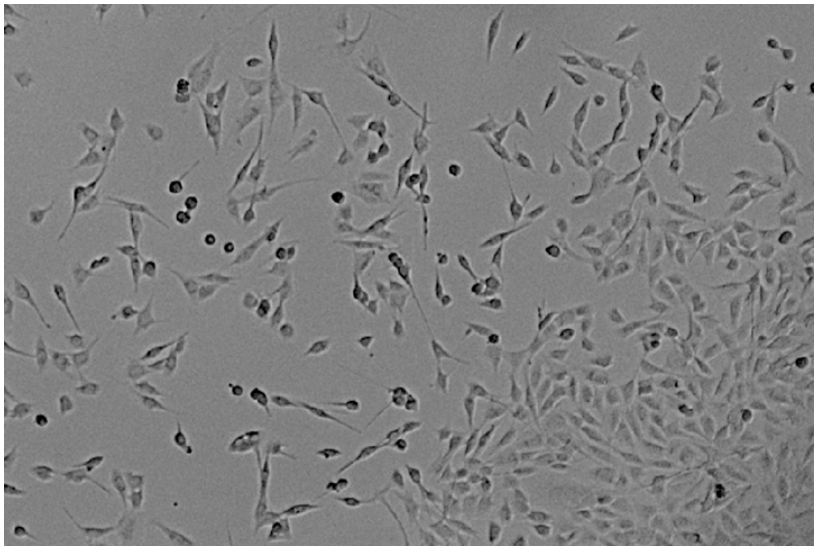

Figure 6: Differentiated C2C12 cells (Source: McGowan institute of regenerative medicine)

4.2 Bio compatibility test

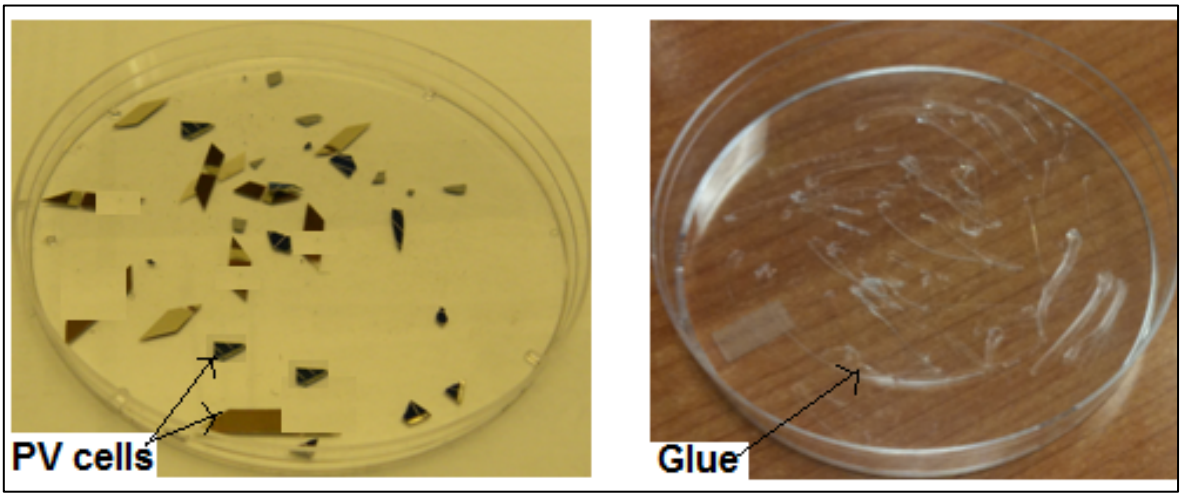

Figure 7: C2C12 cells were cultured with small pieces of PV cells (left) and Glue (right)

Materials mostly used are of certified nontoxic condition from the suppliers. However, for the experiment, we need to verify toxicity and biocompatibility of the PV devices. To conduct the biocompatibility test, biological cells were cultured with sterile pieces of PV cells, and, glue stick in melted form attached with the petridish. During the cell culturing, cell density, and all other protocols were followed as per ATCC. Cells were rinsed with Dulbecco's Phosphate Buffered Saline Solution (DPBS) (Sigma Chemical Inc., St. Louis, MO) and were monitored visually with a microscope to observe the variation in cell morphology. After 4 days of incubation in cell culturing medium at 37^{o}C, and 5% CO_2 cells were observed microscopically for viability. Apart from individual culturing of cells with PVs and glues; cells were also cultured with PV pieces and glues together for biocompatibility test. Figure 4.5 showing the anchorage depended cells maintained their usual sustainability and confluence in the medium along with glue, and PV cells were biocompatible in this investigation.

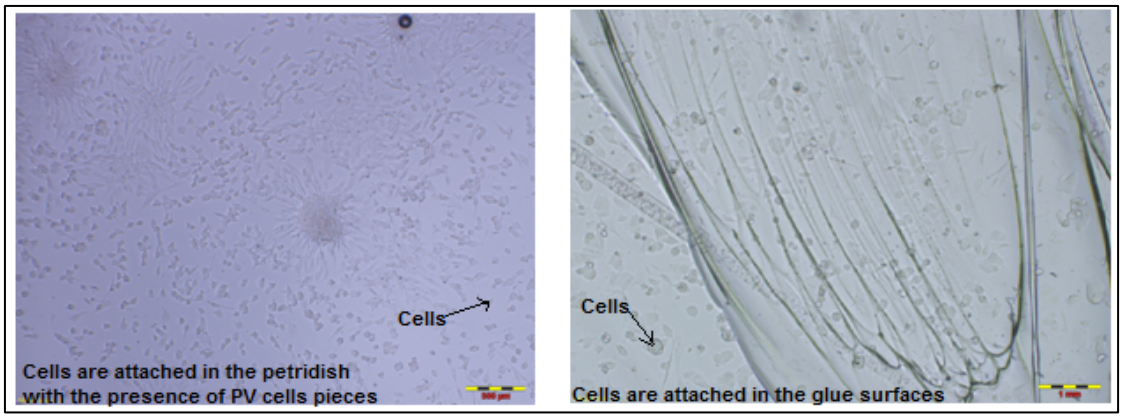

Figure 8: C2C12 Cells are attached and proliferated with PV cell (left) and Glues (right)

4.3 Sterilizing PV cell

Glue walled PV cells were Ultra Violet (UV)/Ozone cleaned for 2.5 minutes to remove surface contamination [1]. Subsequently they were soaked in 70% ethanol overnight and air dried in a sterile ventilated hood. Upon drying, cells were covered with aluminum foil and kept in the dark to remove electrical charge from the PV devices.

4.4 Experimental procedure

The overall experimental procedure is depicted by flow chart in Figure 4.6. The experimental procedure is consisting of two steps: one, culturing cells on PV devices and two,

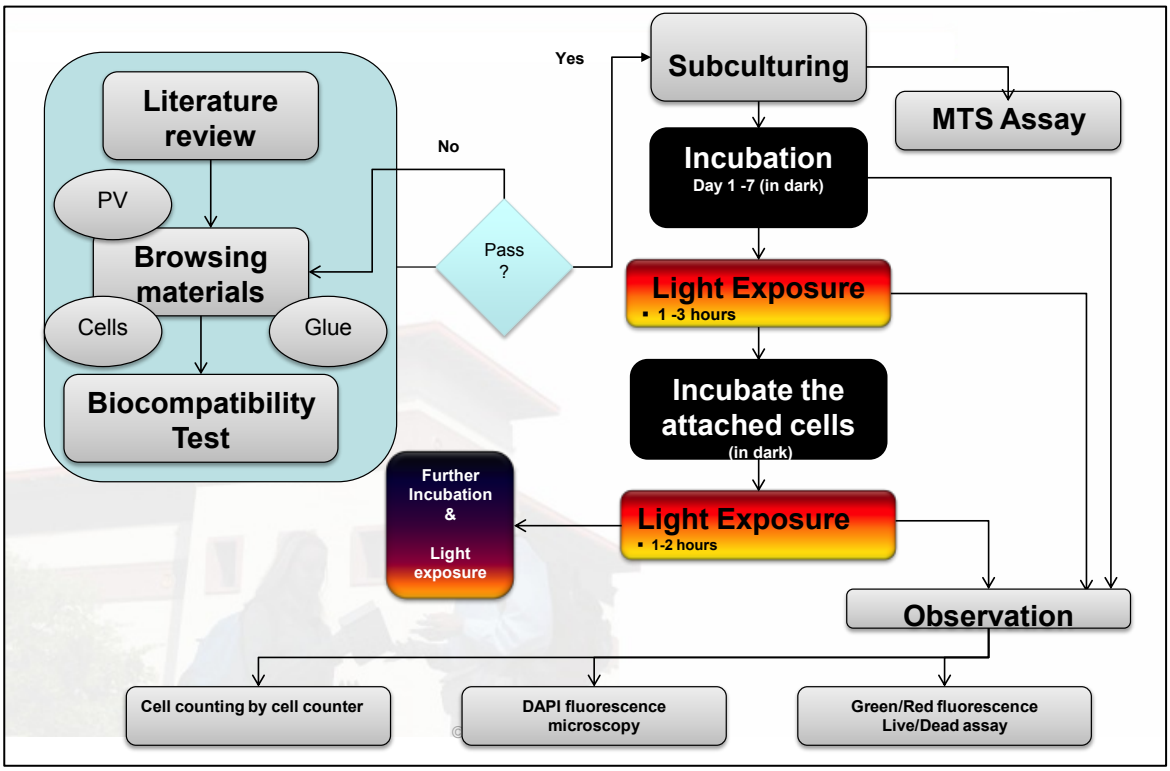

Figure 9: Flow diagram of working procedure

conducting a cell release from the PV devices by exposing them to light. The release study protocol is depicted in Figure 4.7. Cell growth curves were evaluated using an MTS assay (Promega, CellTiter 96, Cat No. G3582) and automated cell counting by Countess® Automated Cell Counter (Life technologies, Cat No. C10227).

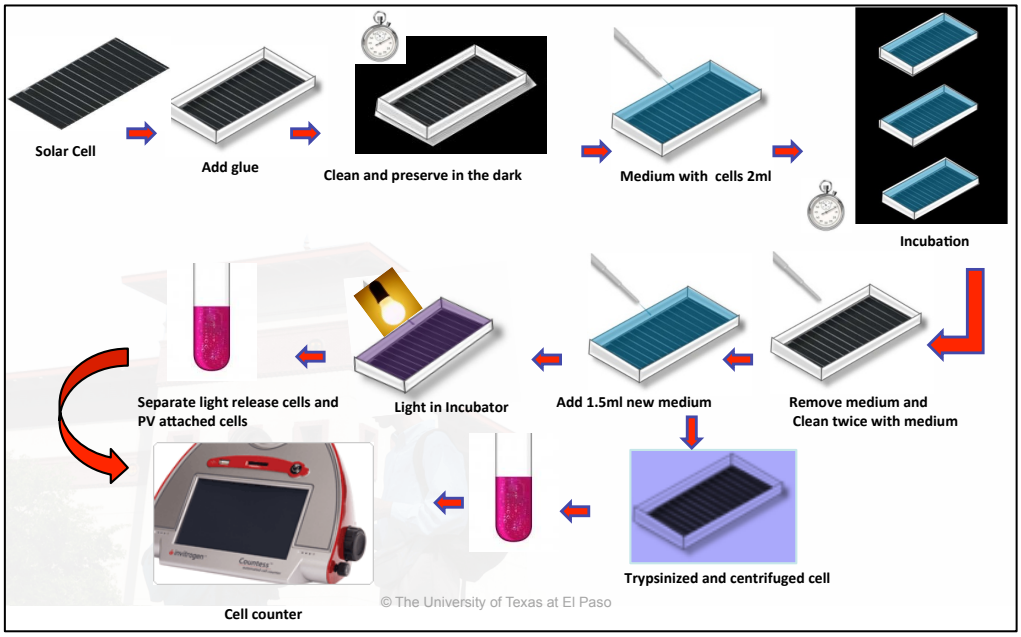

Figure 10: Schematic of experimental procedure

4.4.1 Cell culture

C2C12 myoblasts cell were cultured until 90% confluence, when medium was removed, cells were rinsed and washed with DPBS. Washed cells were trypsinized at with 0.1% trypsin (GIBCO) and incubated for 6 minutes at room temperature. Then they were centrifuged, the supernatant removed, and the cells re-suspended in media. The cell number was measured with a hemacytometer using trypan blue staining [2]. Cells were then seeded at the concentration of 20,000 cells/ PV and incubated for one week. All cell-loaded PV devices were placed in a 150

mm petridish, which was covered with aluminum foil to protect the devivces from accidental light exposure. Every 24 hours, the cells were counted and release experiments performed.

4.4.2 Releasing cells with light effect

Sub-confluent and confluent cell cultures on PV devices were rinsed three times with PBS to clean the cells and remove unattached cells. The devices were exposed to light for 1hour, 1.5 hours 2 hours. The experiments were performed every day for seven days. Light intensity was changed from 200lux to 20000lux. To prevent the cell damage from the heat generated by the light source and to maintain the temperature at 37±2°C the temperature of incubator was lowered to 27°C at the beginning of light exposure. The released cells were collected in the supernatant, centrifuged, and resuspended for counting and other experiments.

4.4.3 Quantification of cells

Cells that adhered to the PV devices after light exposure were rinsed thrice with fresh medium then collected by trypsinizing and employing cell scrapers. The collected cells were centrifuged at 1000 rpm for 5 minutes, the supernatant was moved, and the cells resuspended in fresh medium for cell counting. Cell counting was performed using a Countess® automated cell counter (Life technologies, Cat No. C10227). Trypan blue (0.4%) and the cell suspension were mixed well in 1:1 ratio in small vial. 10µl of blue cell solution was loaded in each cell counting chambers. Single sample measurement provides the live and dead cell concentration/mL, total cell concentration/mL, and viability (% live cells to total cells), and cell size.

4.4.4 Viability evaluation of the cell suspension arrays

The viabilities of the C2C12 cells attached to PV devices and released from the PV devices were evaluated by a two-color fluorescence live/dead assay (LIVE/DEAD® reduced

biohazard Viability/Cytotoxicity Kit #1) and using a solution consisting of SYTO 10 green fluorescent nucleic acid stain dissolved in DMSO and DEAD read nucleic acid stain dissolved in DMSO (Invitrogen, Stockholm, Sweden).. Samples were viewed using a fluorescent confocal microscope Nikon ECLIPSE Ti, and the viability of the cells were evaluated by observing the number of cells stained with SYTO 10 (green) through the method described in section 4.4.4.1, As our cell culturing device PV cells are opaque, 4′, 6-diamidino-2-phenylindole, dihydrochloride (DAPI), a nucleic acid stain was used to visually observe the cell nucleus using an inverted LSM 700 Zeiss microscope and assisted with ZEN2009 software and the procedure of DAPI stained, described in section 4.4.4.2.

4.4.4.1 Cell staining for green/red fluorescent

As our culturing surface is non-transparent, live/dead assay of cell was conducted form centrifuged cell suspension attached on glass cover slip. In case of released cells, centrifuged cells were sub-cultured on 12-well plate and cells were observed directly from the well plate rather cover slip. Remove the supernatant from trypsinzed centrifuged cell suspension to leave the cell pellet only and substitute it with the diluted dye mixture; 200–500 μL should be sufficient to cover the cell pellet.

4.4.4.2 Fixation and staining cell with DAPI

C2C12 monolayers attached to PV surfaces were washed two times or more with PBS, maintaining the level at pH 7.4, and fixed with 3.7% formaldehyde followed by incubation for 5-10 minutes after which formaldehyde was removed, and the cells were rinsed three times with PBS to stop fixation. After rinsing, the nuclei of the cells were labeled with DAPI (Sigma-

Aldrich, 300 n*M*) and incubated for 15-20 minutes. Later the PV surfaces were mounted and observed under an inverted epifluorescence microscope (Zeiss Eclipse E800).

4.4.5 Statistical analysis

Experimental data are presented as means ± standard deviations, with n = 6 for each group of the designated number of separate investigations. Statistical assessment was conducted using unpaired 2-tailed Student's t-tests between experimental group and control group. Rejection null hypotheses by P values less than 0.05 ($p<.05$) were considered significant difference between group. Cells were found in either group under microscopic observation and cell counting machine confirmed a higher level expression of live release cells, compared with control cells that were stuck to PV surfaces.

4.4.6 SEM observation

The fixed samples were imaged using HITACHI S- 4800 field emission SEM utilized electron beam accelerated at 500V to 30kV. Roughly 1cm x1cm sample of PV devices mounted on a sample holder. There was no special surface coating or polishing required for the sample preparation.

Chapter 5: Cell Culture on Photo Voltaic Solar Cells

5.1 Introduction

Cell based therapies are very promising for therapeutic treatment of various diseases and disorders. Cell therapies offer key advantages that include rapid isolation from the host, *in vitro* expansion and delivery to the damaged tissue or organ. Cells in the various forms provide unique potential to customize treatment of injured tissues or to serve as therapeutic agents. These cells can be delivered to the site of injury by [1-3] using scaffolds which also provide mechanical support and serve as substrate for attachment, differentiation, and proliferation [4, 5]. In a particular application, the cells form sheets, which are thin two dimensional (2D) structures, that do not require complicated microvasculature and are easily fabricated, but difficult to handle [6]. Therefore, the better option is single cell delivery from biocompatible substrate that doesn't require special bio processing or complicated handling mechanism. In our research work, we used silicon based PV devices that allowed cells to attach and grow. As PV device is photo or light responsive, electrically excitable cells can be released from the from PV substrate as single cell due to same electrical charge repulsion.

Silicon is widely used material in biomedical devices used in a wide variety of applications ranging from devices for functional electrode stimulation[7], to devices for treatment of Parkinson's disease [8], to electrode-neuron implants [9], and devices for drug delivery [10]. In addition, many micro electromechanical systems devices use silicon materials and are biocompatibile [11]. Fan et al. showed that during implantation of biomedical silicon devices, sufficient cell attachment to the silicon surface is key [12]. To enhance cell adhesion on the silicon surface, Maher et al. [13], and Martinoia [14] coated silicon with polylysine, and

laminin respectively. More cells grew on the coated devices than the silicon alone implants; however, uncoated silicon is best for cell attachment when it is microstructured.

In this paper, we describe the use of commercially available silicon based photovoltaic (PV) devices as substrate for culturing of C2C12 mammalian cells by examining the attachment and growth of the cells. C2C12 is a muscle-like cell line that can form myotubes.

5.2 Material and methods

5.2.1 Materials

5.2.1.1 Silicon substrate preparation

Silicon based photovoltaic (PV) devices that convert the energy of sunlight directly into electricity by the photovoltaic effect were used as silicon substrate for cell culturing. PV cells, 0.8inch X 1.66inch (2cm X 4cm), were obtained from RadioShack® (Model: 276-124). PV devices were prepared to avoid medium leakage as described in [15], adding a nontoxic biocompatible wall made from glue. Glue walled PV cells were Ultra Violet (UV)/Ozone cleaned for 2.5 minutes to remove surface contaminations [16]. Subsequently, they were soaked in 70% ethanol overnight and air dried in a sterile ventilated hood. Upon drying, cells were covered with aluminum foil and kept in the dark to remove any residual electrical charges from the PV devices.

5.2.1.2 Cell culture

Anchorage dependent myoblasts C2C12 mammalian were bought from American Type Culture Collection, ATCC (CRL-1772) grown in Dulbecco's Modified Eagle's Medium (DMEM) augmented with 1% antibiotics, 2mM glutamine, and 20% fetal bovine serum. The pH was adjusted to 7.5. Once confluence was reached the cells were washed with PBS, detached from

petri dish by trypsinizing (.25% trypsin, Sigma Co., St. Louis, MO), counted, and seeded onto the PV devices @ 20,000 cells/cm^2 [17]. The cell cultures were maintained in growth medium and incubated at 5% CO_2, 37°C and 100% humidity changing medium every 24 hours for seven days.

5.2.1.3 Cell detachment from PV

Cells attached to PV devices were very hard to detach from the substrate. After rinsing the cells with PBS, 0.75 ml of trypsin was added to the devices, they were incubated for 5-8 mins and the loose cells were collected. The cells that remained attached to substrates were removed with a cell scrapper and added to the previously collected cells. A total cell count was then obtained.

5.2.1.4 Fluorescence staining with green/ red live dead assay

The cells collected from the devices were centrifuged and the supernatant was replaced by a diluted dye mixture (Component A, Component B, and a FBS as 2:2:1000) and 200–500 μL were placed on top to cover the cell pellet. After 15 minutes incubation of the dye-cell mixture, the solution was replaced with fresh PBS, and then 4% glutaraldehyde was added, followed by 15- 20 min incubation after which the cell suspension was pipetted onto a glass cover slip and observed under epifluorescence.

5.2.1.5 Fixation and staining cell with DAPI

C2C12 cells attached to PV surfaces were washed two times or more with phosphate-buffered saline (PBS), maintaining the pH at 7.4 and fixed with 3.7% formaldehyde for 5-10 minutes. After removing the formaldehyde, the cells were rinsed three times with PBS to stop fixation. After rinsing, the nuclei of the cells were labeled with 0.1μg/ml DAPI (Sigma-Aldrich,

300 n*M*) and incubated for 15-20 minutes. Subsequently, the PV surfaces were washed twice more with PBS. The samples were then mounted and observed under an inverted fluorescence microscope (Zeiss Eclipse E800).

5.2.1.6 SEM Sample preparation

Small sections PV devices of (1cm x1cm) were cut out using a sharp knife and were rinsed with alcohol to avoid any stain or contamination. The samples were then mounted on the SEM sample holder with double-sided tape. There was no special surface coating or polishing required for the sample preparation. The samples were imaged using JEOL JSM-7000F Schottky field emission SEM in combination with Oxford INCA EDS and HKL EBSD systems.

5.3 Result and Discussion

5.3.1 Cell Quantification and viability

An automated cell counter (Life technologies, Cat No. C10227) was used to count live and dead cell concentrations per milliliter (mL), total cell concentration/mL, viability (% live cells to total cells), and cell size. Trypan blue stain (0.4%, AMRESCO, Inc, Tissue Culture Grade) and cell suspensions were mixed well in 1:1 ratio in a cell counting chamber slide. Cell counts are shown Figure 5.1, depicting ta linear rise of cell numbers for the first 5 days, then an abrupt decrease.

The growth of cells in culture proceeded from the lag phase following seeding, to the growth phase, where the cells proliferate linearly. Seeded cells maintained their normal viability and confluence was achieved by day 5. Following confluency, cells in the medium clumped together and the medium produces lactic acid due to deposition of metabolic material waste. Other C2C12 cultures on silicon wafers found confluent cell concentrations of 80,000-

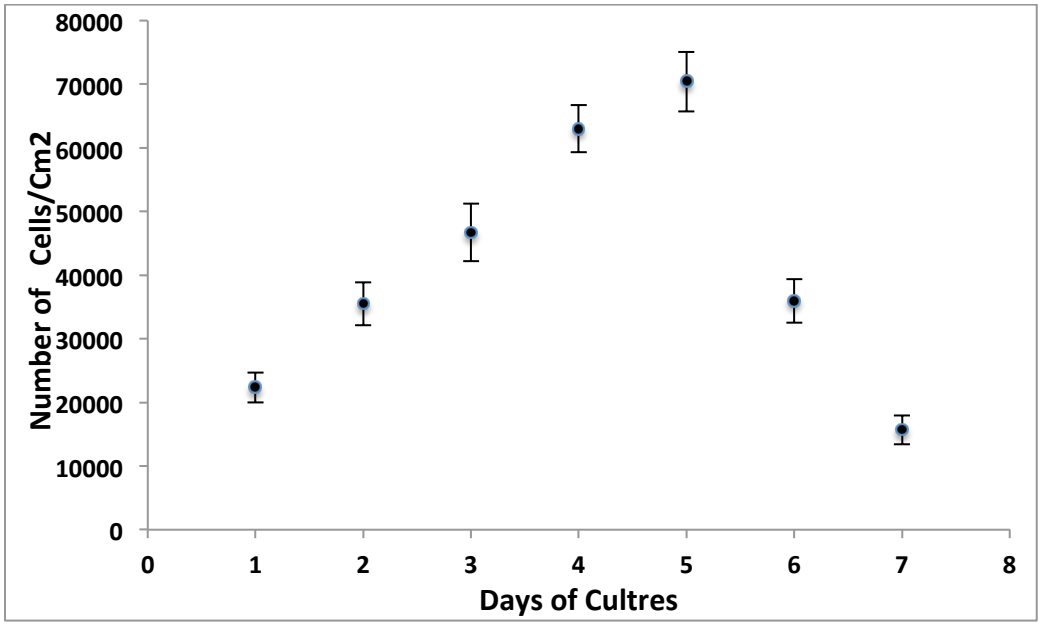

Figure: 5.1 Cell attachment and growth on the PV surface during 7 day of cell culture. Assessment of cell density and viability evaluated using trypan blue and cell counting machine. Data presented the mean± SD, n= 6 .

100,000/sq.cm [12]. However, in our investigation found that maximum cell density on PV devices were 70,000/ sq.cm, which is on the lower end. These cultures were capable of only a limited number of cell divisions, after which they entered a nonproliferative stage. In the cell culture, log phase is missing, because the cell number increased linearly instead exponentially. This may be due to surface finish as our PV devices were without special coating for rapid cell proliferation. They are very susceptible to contamination and overgrowth by microbes.

5.3.2 Light optical microscopy and fluorescence staining with green/ red live dead assay

The C2C12 mammalian cells survived on the silicon surface over more than 7 days after cell seeding. Observation of the cell cultures under the fluorescent microscope shown in Figure 5.2 showed that substantial amount of total proliferated attached cells survived at this time and

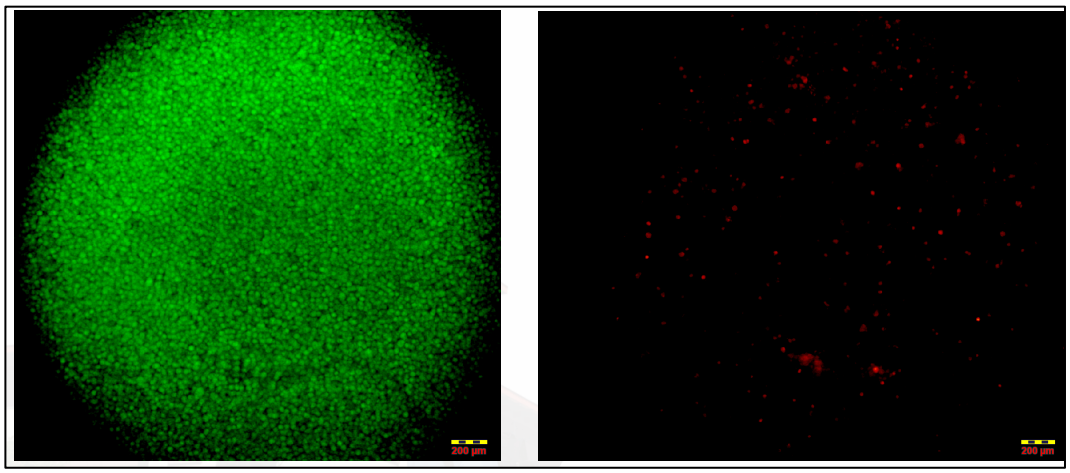

Figure: 5.2: Live/Dead assay of attached cell pellet from PV surface at after 5 day of cell seeding. Cells were trypsinized from the PV surface and centrifuged the supernatant to get the cell pellet. Green /Red fluorescent assay observed by confocal microscope (Density 112000/ml).

attained confluence. The data revealed that the cells could attach and proliferate on the silicon device. The green in the figure indicates live and metabolically active cells while red indicates dead cells.

5.3.3 Cell proliferation by DNA observation

DAPI staining of the attached cells shown in Figure 5.3 also proved that the cells adhered to the silicon devices. Rounded shape of the nuclei is a clear indication of healthy cells and some cells are seen dividing. Thus, the silicon surface characteristics not only support cell attachment but also provide a natural environment for cell proliferation and supporting cell

morphology. It was also noted from the DAPI stain that, while initially the cells were scattered, eventually, they were able to grow and proliferate as indicated by dividing nuclei and the close clustering of nuclei seen in Figure 5.3B and Figure 5.3C.

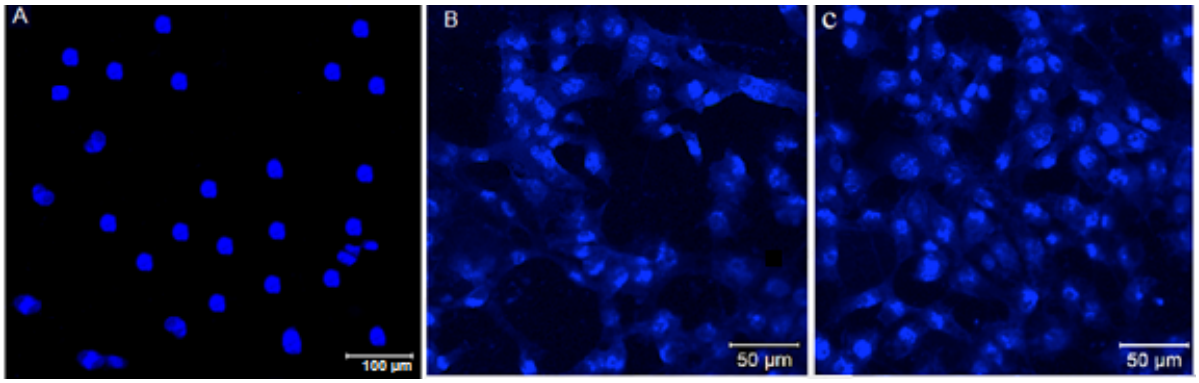

Figure 5.3: 4',6-diamidino-2-phenylindole (DAPI) labeled nucleic acid stain of C212 cell after cell seeding on PV surface at different time period (A) 24 hours of cell seeding , cells are just attached to the surface without proliferation. After cell attachment, cell proliferated with division of nucleolus in Figure B and C. (B) 3 days of cell culture (C) 5 days of cell culture.

It is noteworthy that cell numbers in Figure 5.3 might be higher than those in Figure 5.1. This is most likely due to the fact that a significant amount of the cells is lost during the scraping process or by using trypsin. However, our objective is to demonstrate the cell attachment and growth profile of C2C12 cells on the PV devices during culture.

5.3.4 Microstructure observation by SEM

An SEM image of the resulting PV surface structure is shown in Figure 5.4. The image clearly shows the surface features of the devices. The granular appearance of the device surface indicates that it may be a good substrate for cell attachment, as the roughness may permit seeded cells to anchor better. Cell density of silicon substrate depends on surface roughness and the

uniform roughness observed on our devices could be another reason why our cell densities are

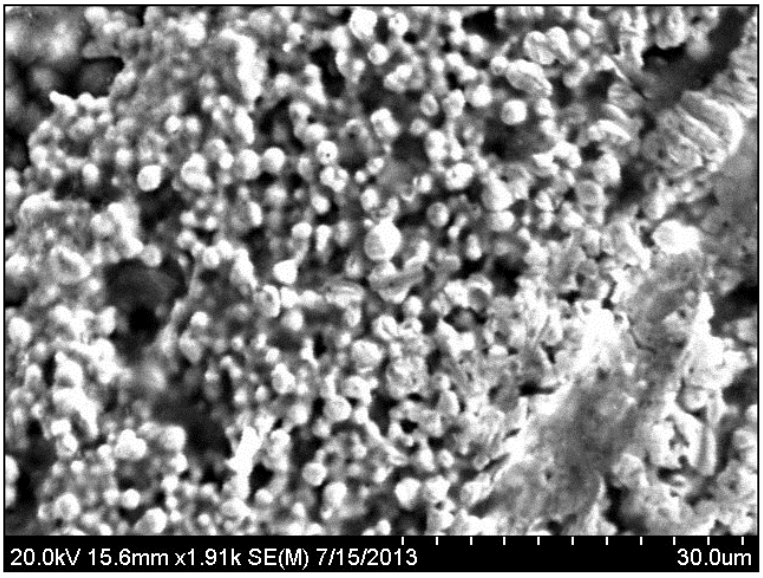

Figure 5.4: Scanning Electron Micrographs (SEM) of silicon PV surface, showing the regular granular microporous silicon surface structure.

lower than the literature values. In order to increase cell attachment, one may coat the surface with cell adhesion proteins. Nevertheless, we have shown that the PV devices can serve as cell substrates or cell carrier physiological conditions that mimic *in vivo* condition [18].

5.4 Conclusions

We demonstrate that porous silicon surfaces of PV devices can be used as cell culturing substrates or scaffold. We also showed that the PV devices are biocompatible and support the growth of mammalian cells but limited the total number of cell even in confluent level. Traditionally, PV cells have been used as a clean renewable source of energy. This study explored the biological applications of silicon based PV devices. However, lot of issues need to addressed to achieve optimum cell growth such surface modifications, or addition of special

coating. Further formulation optimization studies are needed to improve the efficiency of cell attachment and viability. This investigation suggests that microstructured silicon is very promising biomaterials that can potentially be used as cell carrier or vehicle for the delivery of cells and therapeutics.

References

1. Kelm, J.M., et al., A novel concept for scaffold-free vessel tissue engineering: self-assembly of microtissue building blocks. J Biotechnol, 2010. **148**(1): p. 46-55.
2. White, S.A., J.A. Shaw, and D.E. Sutherland, Pancreas transplantation. Lancet, 2009. **373**(9677): p. 1808-17.
3. Sapir, T., et al., Cell-replacement therapy for diabetes: Generating functional insulin-producing tissue from adult human liver cells. Proc Natl Acad Sci U S A, 2005. **102**(22): p. 7964-9.
4. Kaul, G., et al., Failed cartilage repair for early osteoarthritis defects: a biochemical, histological and immunohistochemical analysis of the repair tissue after treatment with marrow-stimulation techniques. Knee Surg Sports Traumatol Arthrosc, 2012. **20**(11): p. 2315-24.
5. Chen, F.H., K.T. Rousche, and R.S. Tuan, Technology Insight: adult stem cells in cartilage regeneration and tissue engineering. Nat Clin Pract Rheumatol, 2006. **2**(7): p. 373-82.
6. Bokhari, M., et al., Novel cell culture device enabling three-dimensional cell growth and improved cell function. Biochem Biophys Res Commun, 2007. **354**(4): p. 1095-100.
7. Chapin, J.K., Impact of neuroprosthetic applications on functional recovery. Prog Brain Res, 2000. **128**: p. 115-20.

8. Gross, R.E. and A.M. Lozano, Advances in neurostimulation for movement disorders. Neurol Res, 2000. **22**(3): p. 247-58.

9. Stanton-Hicks, M. and J. Salamon, Stimulation of the central and peripheral nervous system for the control of pain. J Clin Neurophysiol, 1997. **14**(1): p. 46-62.

10. Desai, T.A., D. Hansford, and M. Ferrari, Characterization of micromachined silicon membranes for immunoisolation and bioseparation applications. J. Membr. Sci, 1999(159): p. 221.

11. Martin Lapisa, F.Z., Göran Stemme, Andreas Gehner and Frank Niklaus, Heterogeneous 3D integration of hidden hinge micromirror arrays consisting of two layers of monocrystalline silicon. Journal of Micromechanics and Microengineering, 2013. **23**.

12. Fan, Y.W., et al., Culture of neural cells on silicon wafers with nano-scale surface topograph. J Neurosci Methods, 2002. **120**(1): p. 17-23.

13. Maher, M.P., et al., Microstructures for studies of cultured neural networks. Med Biol Eng Comput, 1999. **37**(1): p. 110-8.

14. Martinoia, S., et al., A simple microfluidic system for patterning populations of neurons on silicon micromachined substrates. J Neurosci Methods, 1999. **87**(1): p. 35-44.

15. Mohammod Bhuyan, S.A., DANIEL REYNA, JORGE RODRIGUEZ-DEVORA, TAU XU, Targeted Drug Delivery System using Photovoltaic Devices. Advanced Research Journal, 2012. **4**(3).

16. al, R.E.R.e., Removing Surface Contaminants from Silicon Wafers to Facilitate. 47th Annual Technical Conference Proceedings, 2004.

17. Ricotti, L., et al., Quantification of growth and differentiation of C2C12 skeletal muscle cells on PSS-PAH-based polyelectrolyte layer-by-layer nanofilms. Biomed Mater, 2011. **6**(3): p. 031001.

18. Kachouie, N.N., et al., Directed assembly of cell-laden hydrogels for engineering functional tissues. Organogenesis, 2010. **6**(4): p. 234-44.

Chapter 6: A Novel Cell Release System from The Photovoltaic Surface Using Light Source as External Stimulation

Cell therapy is a biomedical technology in which cells are delivered to an injured or diseased organ either individually, collectively as aggregates or sheets, or encapsulated with a scaffold. Some of the delivered cells attach, proliferate, and differentiate and aid in regeneration of the damaged tissue or organ. The effectiveness of the therapy depends amongst others on how controlled the release of cells to the targeted area is. The objective of our study was to investigate the release of cultured cells from silicon based Photovoltaic (PV) surfaces using a light source as external stimulation. C2C12 skeletal muscle cells were cultured on the negative surface of a PV device and upon confluence they were exposed to light and the amount of released cells were quantified as a function light exposure. It was found that light exposure at 25000lux caused significantly cell release from the PV surface. This mechanism may offer an alternative method to release excitable cells without using an enzymatic release method.

6.1 Introduction

In cell expansion, a large amount cells are produced by sub-culturing or splitting from mother cells. A small number of detached cells can then be used to seed a new cultures, while the remainder are used for storage, further processing or cell therapy. For adherent cultures, cells first need to be detached from the culturing surface commonly done with trypsin, EDTA, or a combination of both. Trypsin is an endopeptidase produced by the gastro-intestines of mammals. In cell culture, it is used to permeabilize because it hydrolyzes the membrane peptides. This digestion can result in a substantial amount of cell death if trypsin exposure is too long. In

addition to trypsin, EDTA, a chelating agent is often used to accelerate cell detachment and reduce the harsh effect of trypsin alone. One of the disadvantages of EDTA is the slow action of this agent. The main drawbacks of using enzymatic cell detachment techniques are cost and the necessity of removing the enzyme from the culture after digestion. In addition, enzymatic methods are not applicable for all cells.

Engineering cell release methods without using enzymatic techniques are alternatives that may improve cell detachment without causing damage to cell membrane proteins. Those strategies have in common that they use some external stimulation upon which the cells detach either as single cells, as aggregates or as sheets, Various external stimuli have been used such as magnetic force [1], temperature reduction [2], thermal on-off mechanism on Poly(N-isopropyl acrylamide) (PIPA Am) grafted surface [3], and electro chemical stimulation [4]. Magnetic labeling of cells is very complicated and it can lead to inhomogenious rather than uniform release. Thermal reduction can induce cold stress due to rapid temperature reduction,and this may interfere with normal biological function. Electrochemical detachment requires accurate adjustment electro kinematic forces during the release process, which is difficult to perform.

In our research study, we used a modification of the electrochemical method, where the electric charges are created using silicon based PV devices. The main characteristic of PV devices is their generation of voltage with the light exposure by photovoltaic effect (PVE) [5]. PVE induces negative and positive voltage on n-type region and *p*-type region of the devices [6]. On the other hand action potential based electrically excitable cell such as cardiomyocytes have polarized membranes [7]. We hypothesize that by exposing electrically excitable cells grown on PV devices to light, enough electromotive forces are generated to release some cells from the

culturing surface in a novel non-invasive way. To proof this concept we evaluated the effect of external light on a model cell line, the mouse myoblast cell line C2C12, grown on PV devices.

6.2 Experimental

6.2.1 Preparation of silicon PV cell

Silicon based PV devices in the dimension of 2cm X 4cm were obtained from RadioShack® (Custom assembled in USA, Model: 276-124). A nontoxic biocompatible glue wall was provided surrounding the PV devices to avoid cell culturing liquid medium spillage. PV devices were sterilized exposing to Ultra Violet (UV)/Ozone chamber for 2.5 minutes to remove surface contamination [8]. Subsequently, they glue walled PV devices were soaked in 70% ethanol overnight followed by air drying in a sterile ventilated hood. To conduct experiments, 6 devices were placed in 150mm petridish and fully covered with aluminum foil in the dark to avoid any incidental surface charges on the devices.

6.2.2 Cell culture and light exposure of PV devices.

C2C12 cells were acquired from American Type Culture Collection, ATCC (CRL-1772) grown in Dulbecco's modified Eagle's medium (DMEM) augmented with 1% antibiotics, 2mM glutamine, 10% fetal bovine serum, and the pH was adjusted to 7.5. Confluent cultures washed with PBS, trypsinized (0.25% trypsin, Sigma Co., St. Louis, MO), counted , and the cells were seeded the PV devices at densities of 20,000/cm^2 [9]. The cell cultures were maintained in growth medium and incubated at 5% CO_2 37°C, and 100% humidity. Every 24 hours cells were rinsed with PBS followed by growth medium change as required. Upon confluence of cell culture, PV devices were split in two groups; group 1 for light exposure and group 2 unexposed to light. Figure 6.1 details the cell culture protocols followed. PV devices of group 1 were

exposed to light from a light emitting diode at 700nm and intensity of 2500lux, which corresponded to a voltage generation of 0.35Vdc on the device surfaces. Devices were left under the light source for 1, 1.5, and 2 hours. To prevent cell damage from the heat generated by the light source, the incubator temperature setting was lowered to 26°C 1 hour prior to light exposure and sterile ice cubes were placed under the light and top of the petridish cover to balance local temperature using latent heat of melting. This kept the media temperature at at 37±2°C once the light source was turned on. Any released cells were collected in the supernatant, centrifuged and counted.

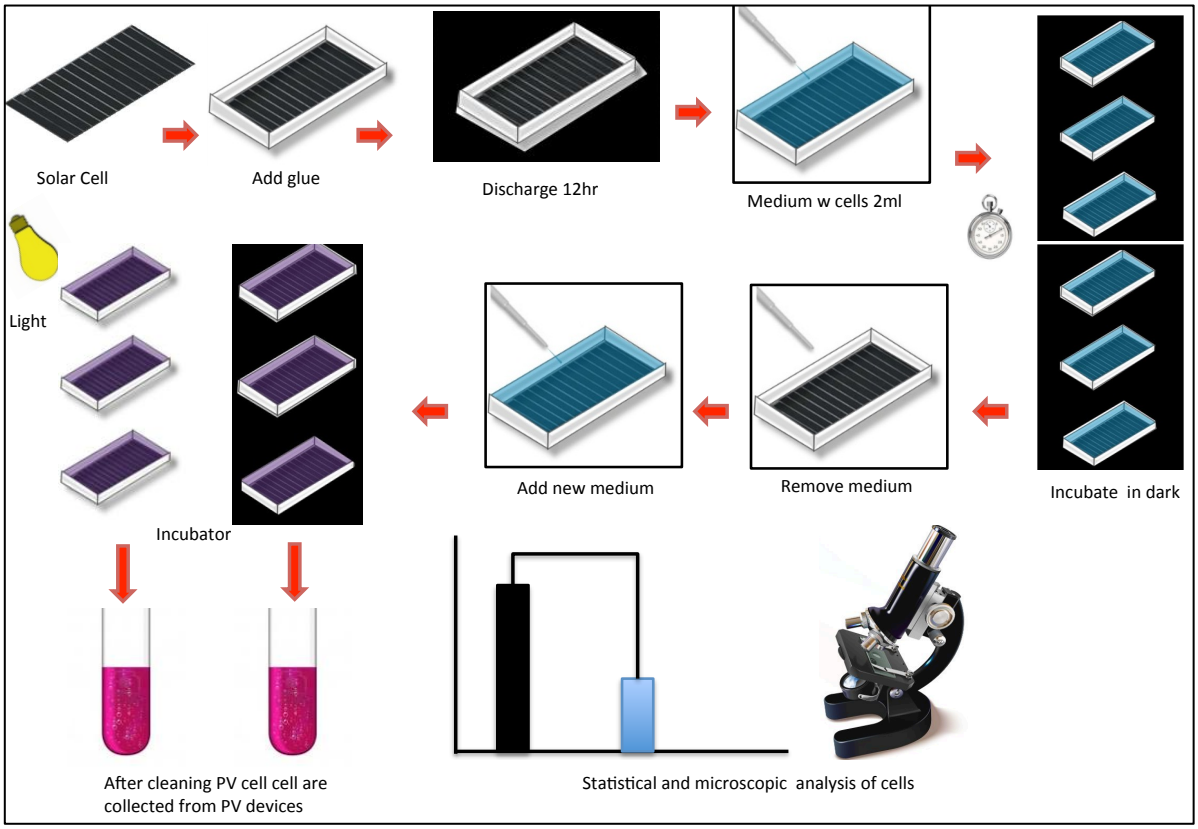

Figure 6.1: Pictorial diagram of cell release by light effect

6.2.3 Fixation and staining cell with (DAPI)

PV surfaces with C2C12 cells were rinsed twice or more with PBS, and fixed with 3.7% formaldehyde for 5-10 minutes. Formaldehyde was then removed by rinsing three times with PBS to stop fixation. The nuclei of the cells were labeled with 0.1μg/ml DAPI (Sigma-Aldrich, 300 n*M*) and incubated for 15-20 minutes. Then the devices were rinsed and observed under an inverted fluorescence microscope (Zeiss LSM 700)

6.2.4 Cell staining for green/red fluorescent:

Mixture of Green/Red dye with PBS (Green: Red: PBS = 1:1:500) added to the cell pellet in a centrifuge tube just to cover the cell pellet and incubated for 15 mins in the dark. Fresh PBS and 4% glutaraldehyde was added to the cell pellet after removing dye mixture and again incubated for 15-20 mins. After thoroughly mixing of the cell pellet with PBS solution in cell suspension, the required amount of stained cell suspension was put on glass cover for Live/ Dead Assay analysis.

6.3 Result and discussion

6.3.1 Cell Release by Light Exposure

Figure 6.2 shows the comparison of cell release by the trypsin and light exposure for 1, 1.5 and 2 hours. We used normal PV devices available commercially for solar power generation. There was no special surface treatment or any protein layer added to the PV devices. Only 46% cells released from the devices using trypsin, the remainder cells were still attached to the devices.. With 1 hour of light exposure maximum roughly 38% of the cells released which is somewhat lower than the number released with trypsin. Also seen from the figure is that less cells released with longer exposure. This is an artifact, which we attribute to decreased cell

viability with increased exposure. This may be due to the fact that the cells were only collected at the endpoint of the experiment. The anchorage dependent C2C12 cells may undergo apoptosis when in suspension for over one hour detaching from culturing substrate or extra cellular matrix [10]. Another potential cause may be damage due to the heat of the light source, although we tried to mitigate this effect as much as possible.

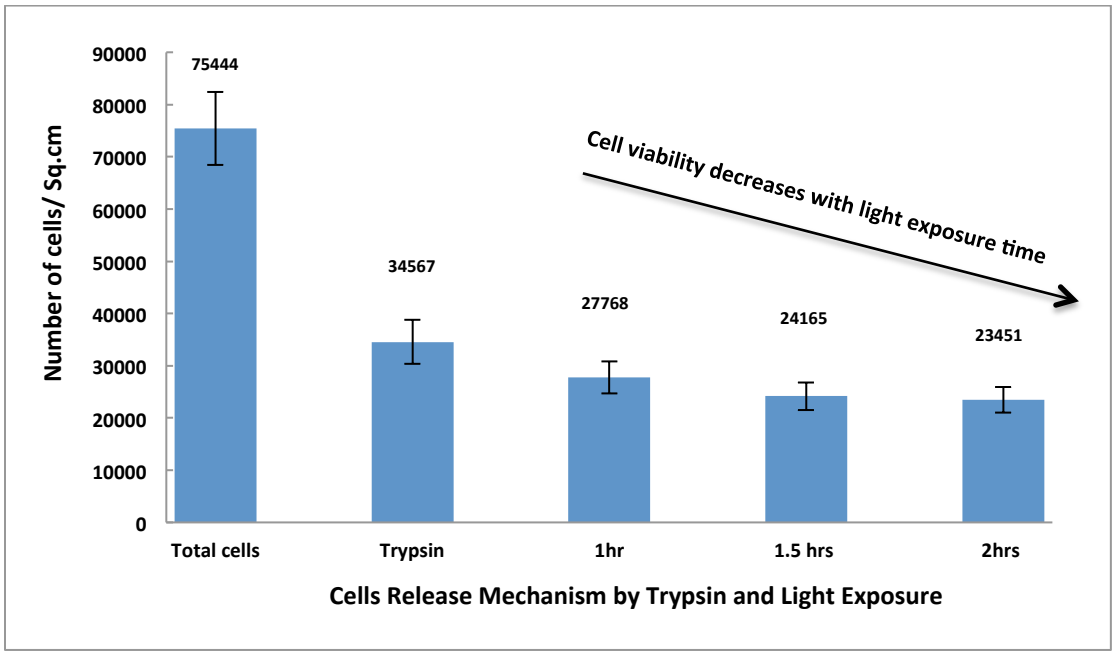

Figure 6.2. Quantification of C2C12 cell released with light exposure, and trypsin after 5 days of cell culture. Cells were released from the PV devices by light exposure at 25000lux. For the light mediated cell release, quantification done only for the live cells.

commercially for solar power generation. There was no special surface treatment or any protein layer added to the PV devices. As a result only 46% cells released from the trypsinization process. Rest of the cells closely attached to the PV surface due to surface properties of PV devices. With light exposure it is shown in the Figure 6.2 that compare to trypsin cell released is

low. With 1 hour of light exposure maximum 38% cell release due to same charge repulsion. Experimental data showed that with more light exposure time cell release remain almost same, however, viability decreases with time. This may be due to anoikis or program cell death caused by anchorage dependent cell detaching from the substrate material. Another potential cause may be damage of heat shock protein due to prolonged light exposure due to temperature rise in culturing medium.

Although we do not know the exact mechanism for the cell release, we propose the following mechanism as shown in Figure 6.3. Light exposure created negative (-ve) surfaces of the PV devices [11] by PVE. When at rest, the potassium ion channels in C2C12 cells are open

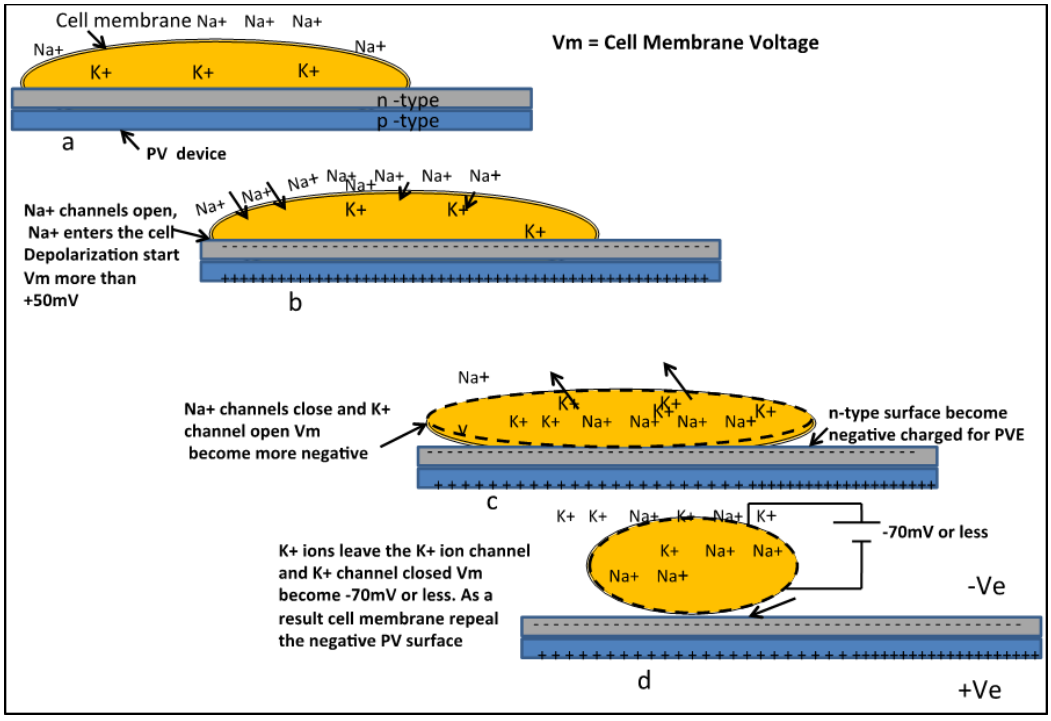

Figure 6.3: Kinetics of cell detachment mechanism for electrically excitable cells from PV devices.

allowing K+ ions to leave the cells for a resting voltage of -15mV [12], [13]. The negative voltage on the PV surface upsets the equilibrium, by either sequestrating K+ ions inside the cells, or by repelling anions in the solution. C2C12 cells have been shown to exhibit hyperpolarization of up to of -75mV when stimulated by extracellular 5′-guanosine-triphosphate (GTP) [14]. We do not know if the surface potential also induces intracellular Ca^{2+} mobilization, but this could be a possibility. The following contraction of the cells may lead to release from the surface.

Other factors that could contribute to cell release in this case is same charge repulsion [15]. Many of the aspects of the proposed mechanism still need to be investigated, however, it is clear that a substantial amount of viable cells are released upon light exposure.

6.3.2 Cell release and retention by nuclei staining

Comparing the two groups, more than 24,000 cell/sq.cm were released in the group exposed to light than from the devices were kept in dark. Consequently, after light exposure PV device should have less number of attached cells in comparison with dark group. Figure 6.4, shows DAPI staining of cells that remained attached to the devices after 1.5 hours of light exposure compared to the control group. On the devices from group1 about 46,000 cells/cm^2 remained in the devices compared to, 72,000 cells/ cm^2 on the control group, which means that roughly 36% of the attached cells were released. These DAPI results compare well with the 38% released cells that were counted in the supernatent. The nuclei in Figure 6.4, lacked regular circular shape, but this may be due to fixation, and permeabilization procedures, which can cause artificial distortion of nuclei [16]. The distinctive clustering behavior of the nuclei may be due to cell division and or too strong attachment of the cells to the PV substrates.

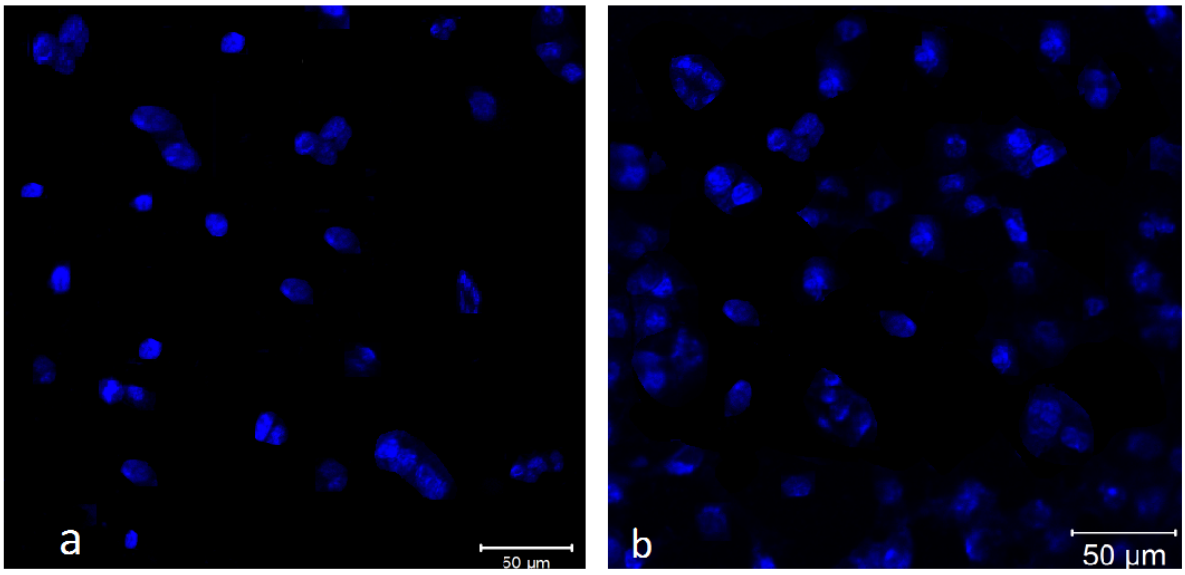

Figure 6.4: Fluorescent microscopy of cell attached PV devices as cell culturing substrates. Round shaped DAPI stain represent cellular nucleolus. In the Figure comparison is shown between two subgroups of PV devices. (a) C2C12 cells from group 1 after 1.5 hour exposure leaving comparatively few nuclei (36). (b) C2C12 cells from group 2 show large number (50) of nuclei per unit area.

Figure 6.5 shows the cell retention capacity of two groups of PV devices. A student t test supported the rejection of null hypothesis that light effect on dark group, and light group is not equal and show a significant (p=0.00014).

Figure 6.6a shows viability of released cells from the PV devices. The image cleary reveas very few dead cells (red) and most of the cells are live (green) resulting in high cell viability. This is an initial indication that the cell membranes remained intact upon release. Figure 6.6b shows a live dead image of reeased cells that were cultured for after 72 hours. Cells were well

attached to the petridish and showed normal phenotype. This indicates that the released cells maintained their normal growth profile and cells could be used further studies.

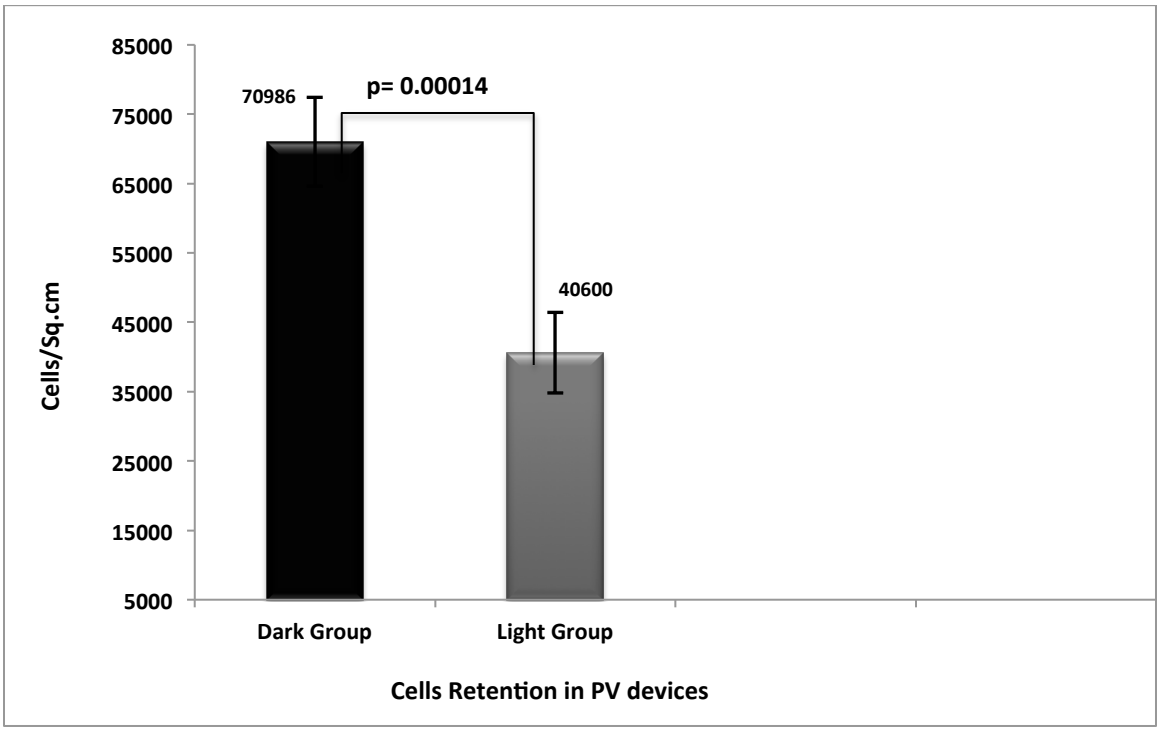

Figure 6.5: Cell retention data was collected from the PV devices and conducted a student t test. For the light group after cell releasing upon light exposure for one hour at 25000lux, remaining attached cells were collected from the PV devices and compared with cells count in the dark group using the Student t test.

Combined, these observations suggest that light could significantly effect on release of C2C12 cells without utilization of trypsin. Device may further be improved by coating with Poly-L-Lysine (PLL) or Bovine Serum Albumin (BSA) [17]. Furthermore, reuse of PV devices reduced the cell attachment and release capacity potentially due to cell membrane protein deposition and erosion of surface.

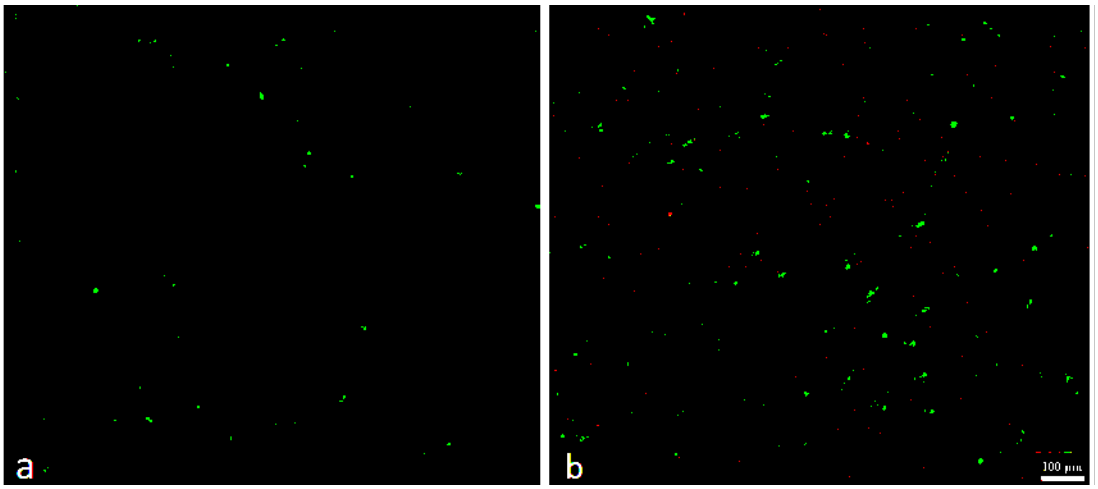

Figure 6.6: (a) Viability/cytotoxicity staining of C2C12 on cell pellet released from light effect. Dead cells are stained in red and live cells are stained in green. The scale bar is 100 µm; (b) C2C12 released by light exposure cultured for 72 hours on 12 wellplate.

6.4 Conclusion

PV devices without surface coatings were used as substrates to culture C2C12 cells. In our experiment we have found that cell attachment on the PV device was 70,000/sq.cm and out of them maximum 38% cells were released by light exposure. To achieve better result modification of PV surfaces such cell adhesive coating with robust cell-repellent agent by light exposure can be used. Our hypothesis was based on the same charge repulsion by cell membrane voltage (V_m) and voltage of PV surface. For electrically excitable cells, V_m depends on transmembrane ionic concentration and gradient that led excitability as well as the depolarization and repolarization. To get the acute result, real time V_m , and ionic concentration need to measure and monitor to correlate with PV voltage. One of the major drawbacks of our pilot study was the selection of light source without heat generation to get designed output voltage 0.5V compare to 0.35V. Also more studies and research are needed to manufacture biocompatible PV devices that

show surface voltage with optimum surface texture compatible with cell culture and release by PVE.

References

1. Kost, J., J. Wolfrum, and R. Langer, Magnetically enhanced insulin release in diabetic rats. J Biomed Mater Res, 1987. **21**(12): p. 1367-73.

2. Nishida, K., et al., Functional bioengineered corneal epithelial sheet grafts from corneal stem cells expanded ex vivo on a temperature-responsive cell culture surface. Transplantation, 2004. **77**(3): p. 379-85.

3. Teruo Okano, N.Y., Hideaki Sakai, Yasuhisa Sakurai, A novel recovery system for cultured cells using plasma-treated polystyrene dishes grafted with poly(N-isopropylacrylamide). Journal of Biomedical Materials Research, 2004. **27**(10): p. 1243-1251.

4. Qi An, J.B., Jurriaan Huskens, Sven Krabbenborg, Jan de Boer, and and P. Jonkheijm*, A Supramolecular stem for the Electrochemically Controlled Release of Cells. Angew. Chem. Int., 2012. **51**: p. 12233 –12237.

5. Califano, F.P.e.a., Current Utilization of Photovoltaic Effect. Chimica & L Industria, 1975. **4**(57): p. 293-293.

6. Yao Y, Z.B., Green MA, Conibeer G, Shrestha SK. . IEEE Phot. Spec. Conf. (2010). Photovoltaic effect in Ge nanocrystals/C-silicon heterojunctions devices. IEEE Phot. Spec. conf (2010), 2010.

7. Gu, Y., et al., High-resolution scanning patch-clamp: new insights into cell function. FASEB J, 2002. **16**(7): p. 748-50.

8. Schwartz, Microscopic solar cells could change cancer therapy. Biophotonics, 2011. **18(1)**: p. 2.

9. Frisch SM, Screaton RA "Anoikis mechanisms". Current Opinion in Cell Biology **13** (5): October 2001, 555–62. doi:10.1016/S0955-0674(00)00251-9. PMID 11544023

10. R.E.R.et al., Removing Surface Contaminants from Silicon Wafers to Facilitate. 47th Annual Technical Conference Proceedings, 2004.

11. Ricotti, L., et al., Quantification of growth and differentiation of C2C12 skeletal muscle cells on PSS-PAH-based polyelectrolyte layer-by-layer nanofilms. Biomed Mater, 2011. **6**(3): p. 031001.

12. Chen, L.J.e.a., Photovoltaic effect in a periodically poled lithium niobate Solc-type wavelength filter. Applied Physics Letters, 2006. **88**(12).

13. Ballaro, B.a.R., P.G., Rotating spiral waves in fertilized ascidian eggs. . Rivista Di Biologia-Biology Forum 2002. **95**(1): p. 101-112.

14. Pietrangelo T. et al., Extracellular guanosine-5'-triphosphate modulates myogenesis via intermediate Ca(2+)-activated K+ currents in C2C12 mouse cells. J Physiol. 2006 May 1; 572 (Pt 3):721-33

15. T. Gonii and B. Hille, Gating of Na Channels, In activation modifier duscriminate among models. Journal of Generl Physiology, 1987, vol. 89 no 2 , 253-274

16. Yeung, T., et al., Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion. Cell Motil Cytoskeleton, 2005. **60**(1): p. 24-34.

17. Mohammod Bhuyan, S.A., DANIEL REYNA, JORGE RODRIGUEZ-DEVORA, Tao Xu, Targeted Drug Delivery System using Photovoltaic Devices. International Journal of Drug Delivery, 2012. **4**(3).

Chapter 7: Photo Responsive Coated Sustainable Novel Petridish for Electrically Excitable Cells

Over more than a century the disposable petridish has become an indispensable lab ware in biological laboratory work for cell culture. Disposable dishes potentially waste a large amount of resources, contribute to an ecological imbalance and add to landfills. Sustainable reusable dishes for culturing cells would be a solution to this problem. We propose to use a reusable petridish-like device based on PhotoVoltaic (PV) devices. The device surface provides an environment for cells to attach, differentiate, and proliferate. Prior investigations revealed, that upon confluence, light exposure to the device surface releases substantial amount of cells by PhotoVoltaic Effect (PVE). Here we show that the remaining cells on device surface could further proliferate to be released later. Direct cell counting and microscopic observation showed that released cells were able to maintain their regular growth profile. Therefore, present pilot study suggests that we made a continuous cell culturing device that may facilitate expansion, screening, counting, and selection.

7.1 Introduction

Cell culture is a series of processes such as cell isolation, cell seeding, and cell attaching on sterile cell friendly surfaces in controlled environment. During culture, cells are seeded on dishes or in flasks to attach, differentiate, and proliferate. Culturing cells or microorganism in a dish that contain liquid growth media would be first recognized by Robert Koch during the growth of bacteria into a colony in the 19th century [1]. Currently used specially coated dishes are shallow cylindrical lidded products made of high quality glass or polystyrene materials. The latter are disposable products and those are widely used in laboratory practices. Culturing cells

on disposable dishes is an expensive and labor-intensive proposition [2-3]. Disposing of these dishes also cost huge amount of money, and natural resources all over the world every year due to intense biological, and medical research.

Modern manufacturing technologies can reduce the cost of disposable dishes, however, the disposing trypsin mediated cell processing equipment remains expensive. In the US alone, 0.22 million tons of disposable plastic labware are produced each year and the associated cost for waste management and recycling is approximately $99.5 million [4]. Recently, researchers have developed highly porous dishes [5] to increase the efficacy of cell processing as well as cell viability. These highly microporous dishes are reliable and robust, and permit many of the manipulation required in cell culture. Drawbacks are the high cost and the incompatibility with some microbial species. Exceptionally porous (40% by volume) aluminum oxide (PAO) coated petridishes can be used as a microbial culture support [6–8]. These devices have nano-range pores (20–200nm) and are capable of retaining cells on their surface. However, these dishes are fragile, not user friendly, and cannot compete on cost. Microtissues Inc., Rhode Island, (Microtissues.Com) invented Scaffold free 3D petridishes by micro mold technology that facilitate scaffold free high cell-cell interactions. The micro-molds are autoclavable and reusable, but cost is very high. Also bioprocessing and storage of single cells is not possible using these dishes. Other advances include the use of very light durable polystyrene dishes, but the disposal and recycling of these remain an issue. Therefore, it is important to develop sustainable reusable petridishes that can be sterilized easily and can be used repetitively.

To address the above issues we investigated sustainable petridishes based on silicon PV devices. The devices are capable of producing electric voltage when they are exposed to visible or infrared light [9], [10]. In our previous studies cells were seeded on PV substrates, and

incubated in incubator to attach, and proliferate. Upon confluence, the devices were exposed to light producing a negative voltage on silicon surface. As result cells detached form the surface, and floated in in the growth medium. The remaining cells were analyzed and appeared normal apart from some minor changes in nuclei shape, which we attributed to fixing and staining artifacts. In this study we will examine if the remaining cells are able will proliferate further and if the release can be performed periodically between periods of growth. .

Trypsin mediated cell release from disposable dishes is widely used. [14-16], Trypsin is a pancreatic serine protease that hydrolyzes polypeptides. Excreted by the pancreas, trypsin has the capability of breaking down proteins, into small peptides [13]. In the cell culture process, trypsin is used to detach adherent cells, by breaking down the proteins responsible for cell attachment [17]. As a result, cells are disengaged from each and the substrate. Trypsin mediated cell detachment is invasive mechanism. During the digestion, , the membrane proteins become damaged and prolonged trypsinization leads to irreversible cell damage. To avoid the harsh trypsin affect, accutase, another cell detachment enzyme, which shows a mixture of proteolytic and collagenolytic activity, is used in laboratory practices [18]. Similarly nonhuman or nonanimal, trypLE Select®is also used to in place of trypsin [19]. Even though these enzymes are comparatively less harmful than trypsin, the use of disposable petridishes and a major concern.

7.2 Materials and methods

7.2.1 Preparation of silicon PV petridish

PV devices that convert the energy of sunlight directly into electricity by the photovoltaic effect were used as cell culturing substrate. Sing crystal structured PV devices made from silicon

(size: 2cm X 4cm) were obtained from electronic retail store RadioShack® (Custom assembled in USA, Model: 276-124). PV devices were prepared to avoid medium leakage adding a nontoxic biocompatible wall made from glue. Glue walled PV cells were UV/Ozone cleaned for 2.5 minutes to remove surface contaminations [11]. Subsequently, they were soaked in 70% ethanol overnight and air dried in a sterile ventilated hood. Upon drying, cells were covered with aluminum foil and kept in the dark to avoid any incidental electrical charges on the device surfaces.

7.2.2 Cell culture

The mouse C2C12 myoblast cell line was purchased from American Type Culture Collection, ATCC (CRL-1772). Cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) augmented with 1% antibiotics, 2mM glutamine, 10% fetal bovine serum, and the pH was adjusted to 7.5. Confluent cells were washed with PBS, trypsinized (.25% trypsin, Sigma Co., St. Louis, MO), counted, and seeded cell on the PV devices at concentrations of 20,000/cm^2 [12]. The devices were placed into a polystyrene dish and maintained in medium and incubated at 5% CO_2, 37°C, and 100% humidity. Every 48 hours cells were replenished with growth medium as per manufacturer's instructions.

7.2.3 Light exposure

Upon confluence of cell culture, and then periodically every five days, the devices were exposed to light at various intensities summarized in Table 7.1, for 1 hour. To prevent cell damage from the heat generated by the light source, the incubator temperature setting was lowered to 26°C 1 hour prior and sterile ice cubes were placed under the dish. This kept the media temperature at 37±2°C once the light source was turned on. Any released cells were

collected in the supernatant, centrifuged and counted. After cell release, the remaining cells on the PV devices were further incubated at 5% CO_2, 37°C, and 100% humidity. Figure 7.1 depicts the schematic sequence of the release experiments.

Table 7.1: Various lights used for PV effect for cell released

Light Intensity	Type of light	Generated voltage (V)	Voltage (mV)/cm^2
2000lux	Fluorescent	0.15	27.8
15000lux	Visible light	0.3	55
25000lux	LED	0.35	64.8
200lux	Infra-red	0.5	92

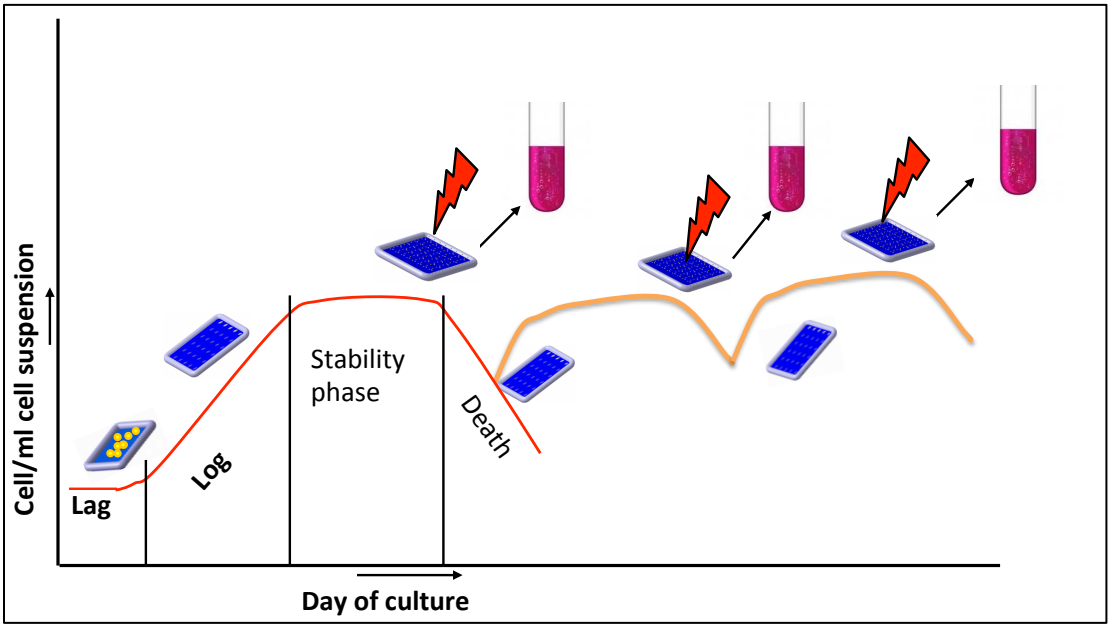

Figure 7.1: Successive cell release mechanism from PV devices upon light exposure

7.2.4 Cell analysis and microscopy

Cell counts were obtained using a Countess® automated cell counter (Life technologies, Cat No. C10227) that allowed comparing cell release, total cells number, and viability of cells at each release experiment. Released cells were collected, cultured on regular dishes and their morphology assessed by optical phase microscopy,

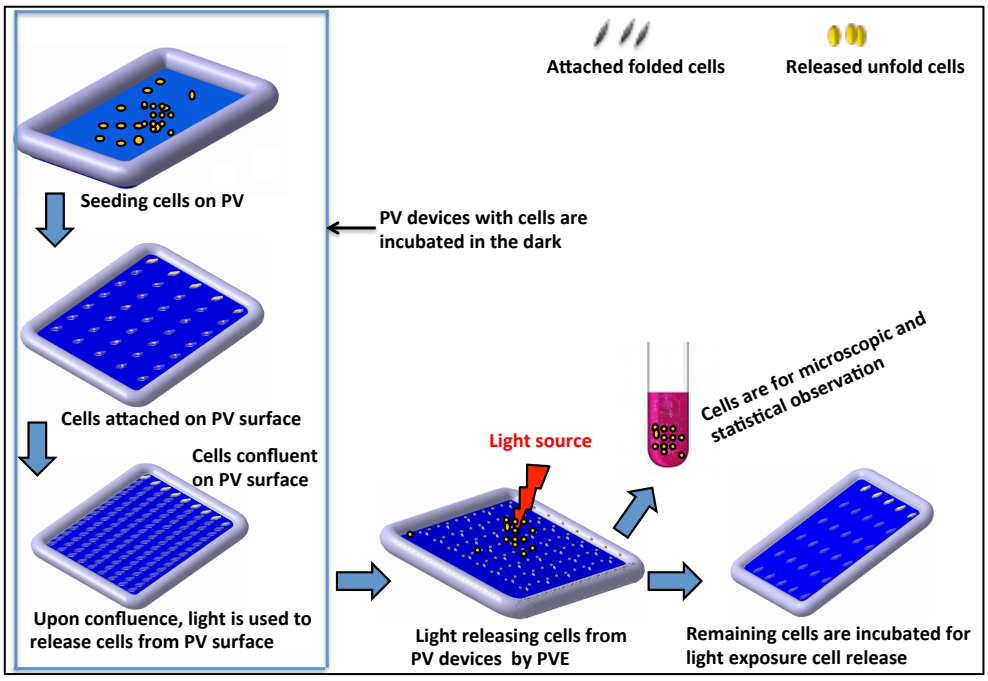

Figure 7.2: Cell culture and release of C2C12 cell from PV devices

7.3 Result and discussion

7.3.1 Cell retention in PV substrate and light exposure cell released

Both enzymatic and nonenzymatic methods can be used for cell release from PV devices. Enzymatic process, for example trypsin is harsh and may damage the cell membrane. In this

study, we used novel non-enzymatic process to release cell from the substrate. Figure 7.3 showing cell release comparison between trypsin, and light. In the both cases a significant amount of cells were released, but, more than 50% cells retained to the PV substrates. The light exposure process is noninvasive process to release cells as it doesn't damage the cell membranes. As a result, the cells that remain attached can easily grow and proliferate further.

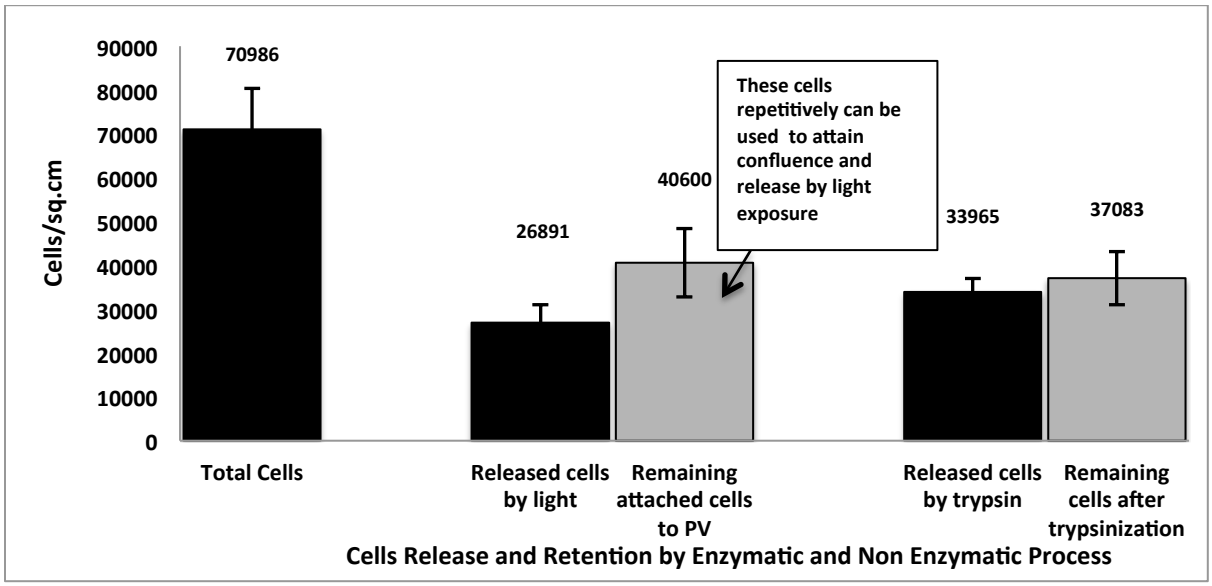

Figure 7.3: Cell release from the PV devices via trypsin and light exposure. Significant amount of ells retained to the PV devices by both enzymatic and non enzymatic process were compared with released cells. Cells count varies from the previous study due to reuse and/or different lot of PV devices.

7.3.2 Successive cell release from PV devices

Figure 7.4 shows cell counts of cells on PV devices as function of time. It was evident from the figure, that cells can be successively released from the devices using light exposure. At day 6, the total number of cells was 70,670 and the number of remaining cells after release was 44,300; the figure also shows the total number of cells released decreases.

In general, the figure shows that after initial seeding, the cells proliferated, and continued to do so for 26 days, when the experiment was stopped. However, we can also observe that the rate of proliferation slows down after each release step. This could indicate that the substrates become increasingly covered with extracellular matrix but it could also be the result of some transformation of the cells. Another potential explanation may be that the devices effect the transmembrane potential of the C2C12 cells on PV substrate, thus affecting mitosis, DNA synthesis, cell cycle progression, viability and metabolism [20-21]. We will study the reason for the decreased proliferation rates later.

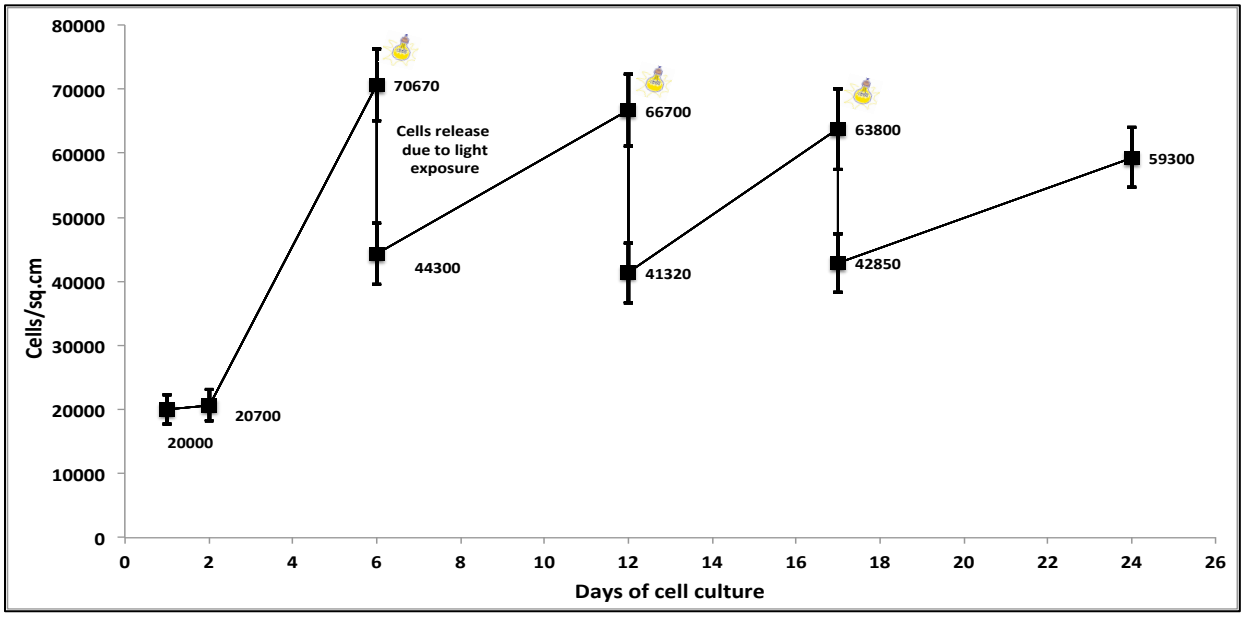

Figure 7.4: To analyze the effects of light on the cell release mouse myoblast C2C12 cells were cultured on the PV devices. Upon confluent in stability phase, cells were release by light exposure (25000lux for 1 hour) and remaining nonreleased cells attached PV were incubated for confluence and successive cell release. At day 6 total number of cells were 70670 at confluent stage and due to light exposure cells were released remaining cells number attached to PV were 44300; therefore total cell released was 26370 (70670 – 44300). Figure also showing the total number of cells decreases at stability phase due to protein deposition on the surface.

As light stimulated process is noninvasive, it didn't damage any cell membrane protein or peptides of the released cells or the cells remaining attached to the devices. In addition, the light does not affect the substrate topography, and as a result, the same device can support continued cell growth and proliferation.

Figure 7.5 shows the relation between the voltage generated by the PV devices and percentage of released cells. These data suggest that the cell release is proportional to maximum voltage generation capacity of the PV device, The highest cell release with acceptable cell viability was for a 1 hour exposure at 25,000 lux; the release rates were higher for a 200 lux IR exposure, but the viability of the released cells was marginal (~20%). Rapid heat generation by the IR light may be responsible for damaging the released and attached cells.. Although we do not know the exact mechanism for the cell release, we propose the following mechanism. Light exposure created negative (-ve) surfaces of the PV devices. When at rest, the potassium ion channels in C2C12 cells are open allowing K+ ions to leave the cells for a resting voltage of -15mV [11], [12]. The negative voltage on the PV surface upsets the equilibrium, by either sequestrating K+ ions inside the cells, or by repelling anions in the solution. C2C12 cells have been shown to exhibit hyperpolarization of up to of -75mV when stimulated by GTP [22]. We do not know if the surface potential also induces intracellular Ca^{2+} mobilization, but this could be a possibility. The following contraction of the cells may lead to release from the surface [2].

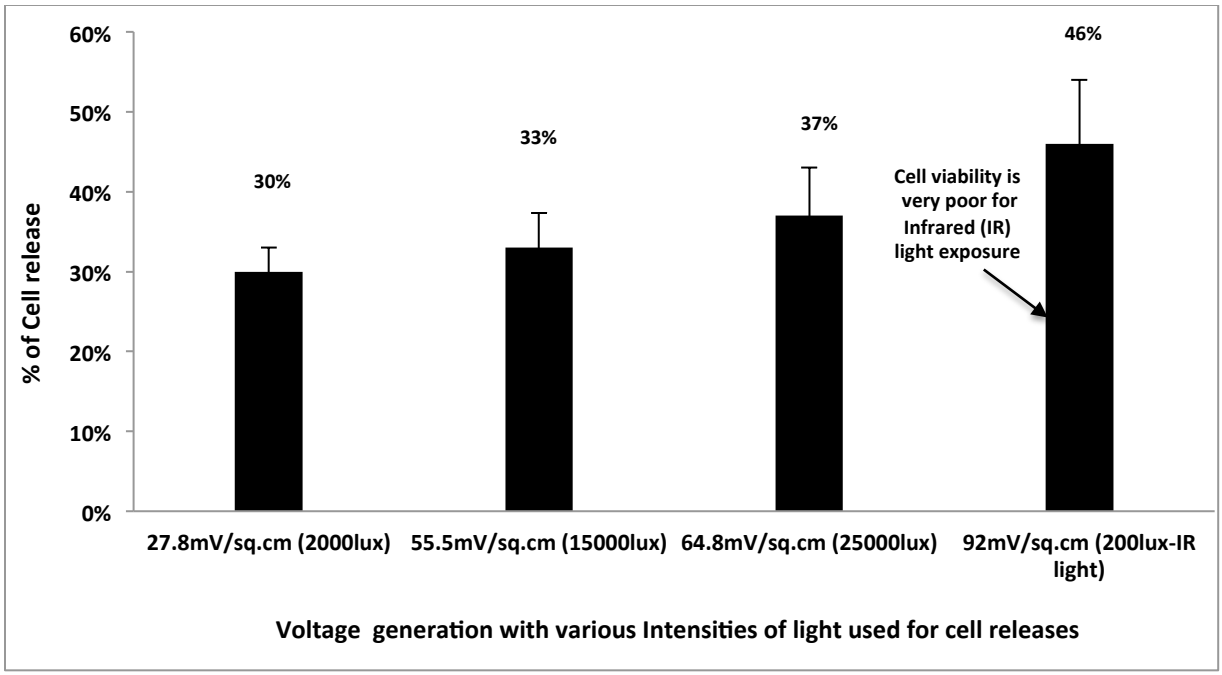

Figure 7.5: Cell release comparison with generated voltage of various intensities of light. For each light source, exposure time 1 hour except for IR light (5 mins). Cell viability was only 20% for IR light.

Figure 7.6 shows morphologies of released cells that were subsequently cultured in regular plates. There are no distinct difference between cells released with trypsin and those released by light exposure. This suggested that cells released by light exposure from PV surface are not transformed and maintain their phenotype.

Full phenotypical characterization of the C2C12 cells still needs to be done and the ability of the released cells to differentiate into myotubes should also be investigated before concluding that the light release technique is as useful as trypsination. Other potential problems for future studies are to measure the effect of the PV devices on the cell membranes by either using voltage-sensitive fluorescent dyes [23] or microelectrode insertion patch clamping [24].

We also plan to conduct Ca imaging studies to verify our release hypothesis. In addition, one could investigate if collagen or fibronectin coating of the devices will improve cell proliferation after successive releases.

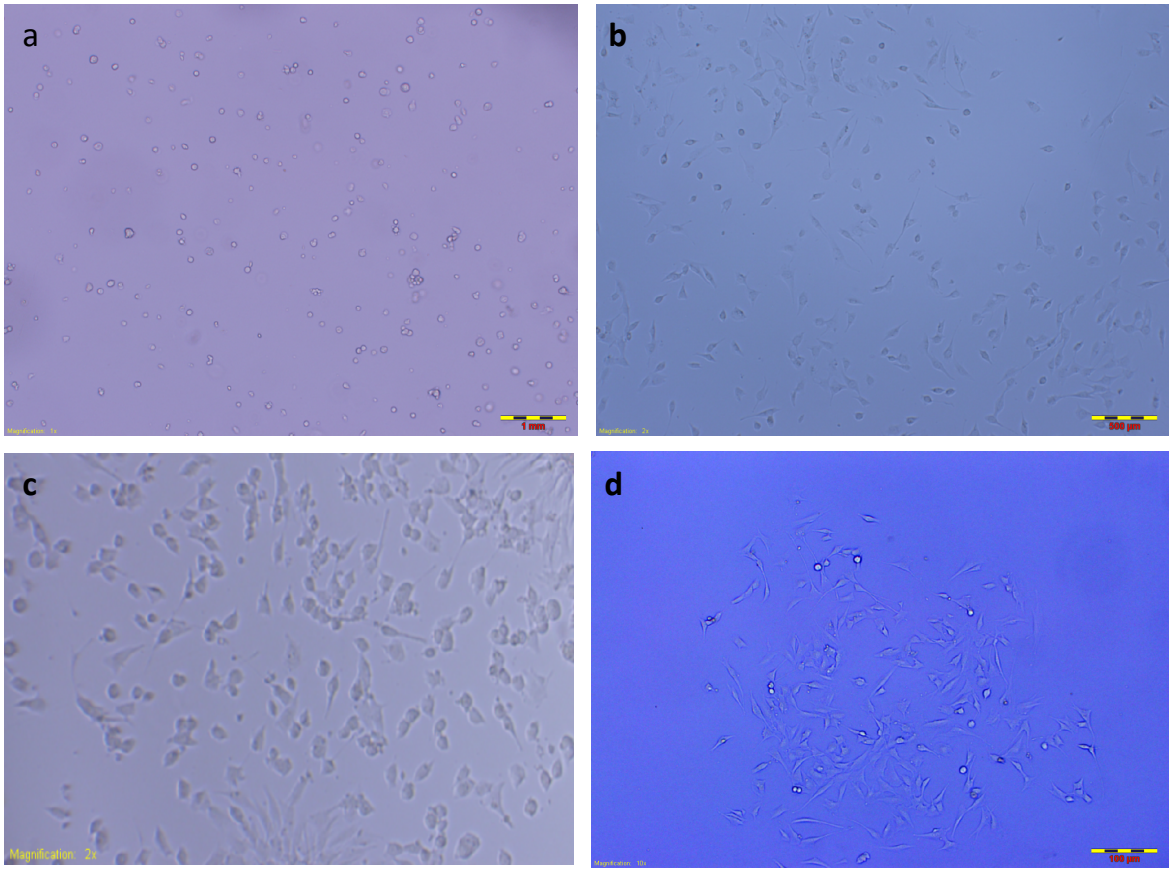

Figure 7.6: To investigate whether the light stimulated released cells can maintain their viability such as attachment, and proliferation, C2C12 cells were seeded in 6-well plates with DMEM and incubated in a incubator for optical microscopic observation. (a) Cell attachment after 6 hours of cell seeding. (b) 24 hours of cell culture. (c) 48 hours of cell culture. (d) 72 hours of cell culture.

For over a decade global leaders were focusing on developing green technology including green manufacturing process to reduce pollution and dependency on natural resources. Biomedical engineering is rapidly growing field and a growing part of the global economy

Reusable cell culture substrates, as those investigated here, could paly an important role in replacing disposable culture dishes without utilization cell unfriendly trypsin. Although the usage of disposable dishes seems economical, using trypsin in cell processing is time consuming and tedious process and the cost of disposing are still very high. Reusable sustainable culture dishes will, in the long run, save energy, raw materials and disposing costs. As the total land, and natural resources of the world is limited, production and disposal waste management practices affect a large regional population. Using reusable petridish will prevent environment pollution such as reduce usage of energy, water, solid waste and eventually total manufacturing costs [25].

7.4 Conclusion

We have developed an environmentally sustainable and less hostile methods for culturing and subculturing C2C12 cells that is a novel way of releasing cells for analysis, including cell delivery.. Using PV devices, we have shown that 37% of attached C2C12 cells could be released upon light exposure. The remaining cells continue to grow until a new release episode. This continuous growth and release was repeated for 30 days with only minor slowdown in growth rates. The released cells showed normal morphology. In addition to being a reusable, environmentally friendly system, the idea of a continuous cell culture that does not involve bioreactors is attractive and may open the door to many fields in which large expansion of cells are needed.

References

1. Carter KC (1987) Essays of Robert Koch (Greenwood, Portsmouth, NH).
2. Gilbert, P.M. et al., Substrate elasticity regulates skeletal muscle stem cell self-renewal in

culture, Science 329 (2010), pp. 1078–1081.

3. Chowdhury, F. et al., Soft substrates promote homogeneous self-renewal of embryonic stem cells via downregulating cell-matrix tractions, PLoS ONE 5, e15655 (2010).
4. William A. Rutala and Glen Mayhall, Medical Waste, The Society for Hospital Epidemiology of America, 1992
5. Colin J. Ingham et al.,The micro-Petri dish, a million-well growth chip for the culture and high-throughput screening of microorganisms, Proceeding Of the National Academy of Sciences of USA, 2007.
6. Ingham CJ, van den Ende M, Pijnenburg D, Wever PC, Schneeberger PM (2005) Appl Environ Microbiol 71:8978–8981.
7. Ingham CJ, van den Ende M, Wever PC, Schneeberger PM (2006) J Med Microbiol 55:1511–1519.
8. Ferrari BC, Binnerup SJ, Gillings M (2005) Appl Environ Microbiol 71:8714-8720.
9. Califano, F.P.e.a., Current Utilization of Photovoltaic Effect. Chimica & L Industria, 1975. **4**(57): p. 293-293.
10. Yao Y, Z.B., Green MA, Conibeer G, Shrestha SK. . IEEE Phot. Spec. Conf. (2010). Photovoltaic effect in Ge nanocrystals/C-silicon heterojunctions devices. IEEE Phot. Spec. conf (2010), 2010.
11. R.E.R.et al,., Removing Surface Contaminants from Silicon Wafers to Facilitate. 47th Annual Technical Conference Proceedings, 2004.
12. Ricotti, L., et al., Quantification of growth and differentiation of C2C12 skeletal muscle cells on PSS-PAH-based polyelectrolyte layer-by-layer nanofilms. Biomed Mater, 2011. **6**(3): p. 031001.

13. Chen, N.et al., Designing Protease Sensors for Real-time Imaging of Trypsin Activation in Pancreatic Cancer Cells , Biochemistry 48, 3519, 2009
14. chuchert-Shi et ail,Peptic and tryptic digestion of peptides and proteins monitored by capillary electrophoresis with contactless conductivity detection. , Anal Biochem 387, 202-7, 2009
15. Soleimani, M., and Nadri, S.: A Protocol for Isolation and Culture of Mesenchymal Stem Cells from Mouse Bone Marrow , Nat Protoc 4, 102, 2009
16. Banumathi, E.et al., High-yielding Enzymatic Method for Isolation and Culture of Micr vascular Endothelial Cells from Bovine Retinal Blood Vessels , Microvasc Res 77, 377, 2009
17. Trypsin-EDTA (0.25%)" Stem Cell Technologies. Retrieved 2012-02-23.
18. Bajpai R, Lesperance J, Kim M, Terskikh A V. Efficient propagation of single cells accutase-dissociated human embryonic stem cells. Mol. Reprod. Develop. 2008;75:818–827.
19. Trond Aasen & Juan Carlos Izpisúa Belmonte, Isolation and cultivation of human keratinocytes from skin or plucked hair for the generation of induced pluripotent stem cells, Center of Regenerative Medicine in Barcelona, Barcelona, Spain, February 2010; doi:10.1038/nprot.2009.241
20. Bortner, C. D., & Cidlowski, J. A. (2004). The role of apoptotic volume decrease and ionic homeostasis in the activation and repression of apoptosis. Pflugers Archiv, 448, 313–318.
21. Franco, R., Bortner, C. D., & Cidlowski, J. A. (2006). Potential roles of electrogenic ion transport and plasma membrane
22. Pietrangelo T. et al., Extracellular guanosine-5'-triphosphate modulates myogenesis via intermediate Ca(2+)-activated K+ currents in C2C12 mouse cells. J Physiol. 2006 May 1;572(Pt 3):721-33
23. Loew, L. M. Voltage-sensitive dyes: Measurement of membrane potentials induced by Dc

and AC electric field Bioelectromagnetics, 1992, (Suppl 1):179–89.

24. Ling, G, & Gerad, R.W., The normal membrane potential of frog sartorius fibers. Journal of Cellular and Comparative Physiology, 194934, 383–396.

25. S. L. Hart, “A natural resource-based view of the firm,” Acad. Manage. Rev., vol. 20, no. 4, pp. 986–1014, 1995.

Curriculum Vita

Mohammod Bhuyan achieved his Bachelor degree in Mechanical Engineering from Bangladesh University Engineering and Technology (BUET) in 1998. In order to peruse higher studies, he came to USA in 2001, and received his Master of Science degree in Mechanical Engineering and Material Science in 2004 from Tuskegee University, Alabama. His previous excellent research, industry skills, and critical thinking enabled him enter into bioengineering project on development of novel photovoltaic devices for targeted delivery of biomolecules and livings cells. The research focus in Biomedical laboratory was on the design of therapeutic cells delivery for the degenerative treatment. Mr. Bhuyan's exceptional research ability and contributions are highly valued by his colleagues, researchers, and biomedical societies. In 2013 he was elected general secretary and treasurer of student chapter of Society for Biomaterials for term 2013-2014. Dr. Bill Tseng, and Dr. Thomas Boland supervised his dissertation, Cell Releasing System Using Light Responsive Photovoltaic Devices. He is currently working at Baxter Healthcare as Test Method Validation Engineer on Biomedical Devices.

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