

Myoblast Transplantation for Cardiac Repair Using Transient Immunosuppression

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Abstract

Myoblast transplant for cardiac repair offers an alternative therapeutic modality for cardiac repair. This novel cell based approach has the potential for replacing cardiomyocytes damaged during the disease process. Studies in the small and large animals and human phase-I trials have shown that myoblast transplantation into damaged myocardium results in the improved myocardial function and hemodynamic parameters. However, many aspects of myoblast transplantation such as the mechanism by which myocardial function improves and the fate of the transplanted myoblasts remain unclear, shrouding the success of myoblast transplantation therapy. The survival of the donor cells post transplantation is an obstacle to overcome. We studied the fate of human myoblasts xenograft carrying therapeutic and reporter genes in porcine heart models of chronic ischemia and infarction with encouraging results. The salient feature of our study was the long-term survival of the xenomyoblasts for up to 7 months of observation using a minimal dose of 5mg/kg cyclosporine transiently. The immunosuppression starting 5 days prior to and continuing for six weeks post cell transplantation effectively supported the long term survival of the xenomyoblasts carrying exogenous genes. Histochemical and immunohistochemical data revealed no infiltration of host immune cells at the site of xenograft. Furthermore, the transplanted cells contributed towards improvement in hemodynamic parameters and global cardiac performance. We conclude that transient immunosuppression using cyclosporine is effective for successful myoblast therapy for cellular cardiomyoplasty.

Key words: human myoblasts, immunosuppression, porcine, transient, xenograft.

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Left ventricular dysfunction in a failing heart is primarily due to extensive cardiomyocyte loss [23]. The problem is further accentuated due to the limited capacity of the myocardium to regenerate in the event of injury due to the absence of satellite cells and lack of DNA telomeric repeats in the cardiomyocytes. Cellular therapy for myocardial reconstruction using myogenic cells exploits their intrinsic repair ability and has emerged as a promising approach in cardiovascular therapeutics [12, 34]. Cells from various sources have been used for this purpose including fetal and adult cardiomyocytes, bone marrow stem cells and embryonic stem cells [9, 31, 37, 38]. In terms of application, however, skeletal myoblasts have superior characteristics and desirable traits (Table I). The aim of myoblast transplantation is to provide sufficient number of the myogenic cells which may compensate for the cardiomyocyte loss [8]. Further more, these cells are expected to differentiate into myotubes and form muscle fibers that replace the scar. Myoblast transplantation results in

stable grafts and improved global cardiac performance [4, 7, 21, 32, 33, 44, 52]. The methodology however, is far from been optimized and is fraught with problems, the most significant of which is the profound and acute cell death in the initial phase post-transplantation related with host immune response [11, 42].

It is well known that the organ and cell transplantation between different individuals of the same species is poorly tolerated without immunosuppression. Xenotransplantation is even more problematic with rapid rejection if transplanted without immunosuppression. Immunity to xenograft is often thought to be difficult to deceive as compared to allografts due to the fact that all individuals have a strong innate immune mechanism comprised of xenoreactive antibodies, complement system and natural killer cells. Further to this, xenografts usually carry a diverse set of antigens which may trigger a stronger response. This phenomenon is evident even with the use of autologous cells if they are expanded for too long in vitro due to the expression of neo-antigens [41]. However, re-

Human myoblasts for cardiac repair

Table I. Advantages of skeletal myoblast transplantation for cardiac repair.

- Safety of the skeletal myoblast transplantation.
- Ability of undifferentiated myoblast amplification in vitro.
- Skeletal muscle cells are more resistant to ischemia than the cardiomyocytes.
- There are no availability problems and ethical issues involved in their use.
- The use of autologous skeletal myoblast circumvents the problem of graft rejection and alleviates the need for immunosuppression.
- Continued proliferation when grafted in injured myocardium. The in-pot of small number of cells ultimately develop in to large grafts.
- The grafted myoblasts may establish some satellite reserves to counter subsequent injury.
- There is no probability of unbridled growth as the proliferation of skeletal myoblast in vivo has been shown to stop after about one week of transplantation.

cent reports from various research groups and our own experience with myoblast transplantation in porcine heart models of chronic ischemia and infarction have generated some interesting data. Leichty and coworkers have documented implantation of bone marrow derived human mesenchymal stem cells into the fetus of the sheep [30]. The cross-species cellular graft survived for up to 13 months in the host tissue without immunosuppression. Even more intriguing results have come from Saito and colleagues [47]. They have managed xenograft of MHC-I and II expressing mesenchymal stem cells derived from mice bone marrow into rats. The donor cells have not only survived in the immunocompetent hosts, rather they were recruited to repair injured myocardium. These reports highlight the immuno-privileged nature of the mesenchymal stem cells.

A similar report has documented mouse embryonic stem cells for transplantation into immunocompetent rat heart thus exploiting the immuno-privileged nature of the embryonic stem cells [37]. The same researchers have co-transplanted human fetal cardiomyocytes and human mesenchymal stem cells in a model of myocardial infarction [36]. The human cell xenograft survived for up to six weeks in a porcine host. Donor specific tolerance was achieved by immunosuppression using 15mg/kg daily dose of cyclosporine. Our experience in porcine heart models of chronic ischemia and infarction has revealed the feasibility and effectiveness of cyclosporine administration for transplantation of human myoblasts [7]. A salient feature of our study is the long term survival of the donor myoblast xenograft in the host myocardium using transient cyclosporine immunosuppressive therapy at the time of myoblast transplantation. Our results reveal the 'conditionally' immuno-privileged nature of human myoblasts. The use of human myoblasts expressing *lac-z* reporter gene enabled us to assess histologically the in

vivo fate of the donor cells post transplantation. The xenomyoblasts were observed for up to 7 months post transplantation in the host tissue.

Human Skeletal Myoblasts

Skeletal myoblasts are the precursors of the skeletal muscle (Figure 1). Some of the important characteristics of myoblasts are listed in Table I. Unlike cardiomyocytes, they are capable of extensive mitosis without any loss of myogenicity or development of tumorigenicity. In response to injury, myoblasts have the intrinsic ability to re-enter cell cycle and give rise to two types of the cell populations [56]. One of these after achieving fusion competence through the expression of the surface proteins integral to the myoblast fusion, fuse and differentiate into myotubes. These myotubes are subsequently integrated into the muscle architecture and deposit actin, myosin, troponin and tropomyosin that eventually organize into sarcomeres, the structural units of muscle contraction. The other progeny of myoblasts leaves the cell cycle and forms new satellite cells found between the basement membrane and the plasma membrane of every skeletal muscle fiber.

The purity of myoblast preparation before transplantation is imperative. A common pitfall of myoblast culture is fibroblast contamination. From previous dose response studies in muscular dystrophies, it is estimated that the dose of one billion pure myoblasts is optimal to produce the regenerative heart. This conjecture still has to be tested in future studies.

The contaminating population of the cells in the myoblast preparation is one of the factors that trigger the cell graft rejection post transplantation. Human myoblasts during the present study were manufactured according to Cell Therapy Inc., Singapore SOP's with a license of the U.S. Patent No. 5,130,141 [28]. The biopsy site on the rectus femoris of the donor was injured by repeated puncture under local anesthesia. Three days later, 3-5 g skeletal muscle biopsy was obtained from the injured site and

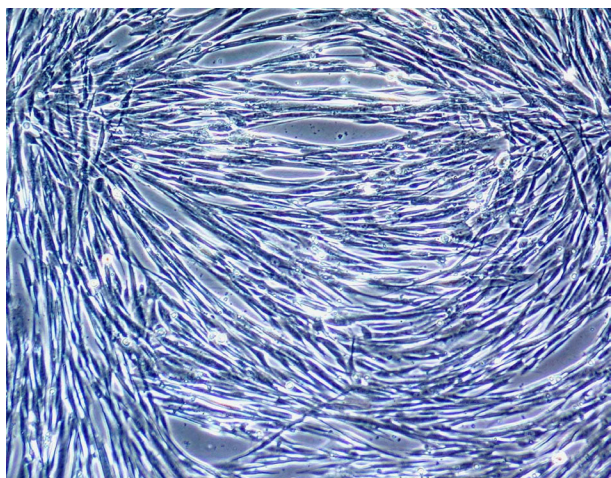


Figure 1. Phase contrast photomicrograph of human myoblasts culture at 72 hours after seeding (magnification = 200x).

Human myoblasts for cardiac repair

processed by the patented procedure to generate myoblasts. The cells were expanded in 225mm² tissue culture flasks (5x10⁵ cells/flask) pre-coated with collagen using patented Super Medium at 37°C and 5% CO₂ until confluent. Myoblasts were >95% pure as determined by desmin staining (Figure 2). For donor cell identification post-transplantation, the cells were labeled with *lac-z* reporter gene using retroviral vector carrying *lac-z* reporter gene. As a monolayer culture, they were incubated with supernatant from FLY-A4 cells containing 1x10⁶ viral particles/ml for 8 hours. Transduction procedure was repeated three times at 24 hour interval.

Our optimized multiple transduction method yielded highly efficient 75-80% *lac-z* positive cell population (Figure 3). Dye exclusion test using trypan blue revealed over 95% cell viability at the time of injection. The study was conducted with a license of the Singapore Patent No. 34490 (WO 96/18303) [29]. Our present study is the first ever attempt to assess the in vivo behavior of donor human myoblasts as a graft in animal models for cellular cardiomyoplasty.

Animal Models

Various organ xenotransplantation models have been studied and applied clinically with little success; models of cellular xenotransplantation have been less well studied [5, 51, 55]. The successful creation of a xenotransplantation model for human myoblast transplantation into the heart allowed the study of human cells in pathophysiological environment such as ischemia or infarction. This

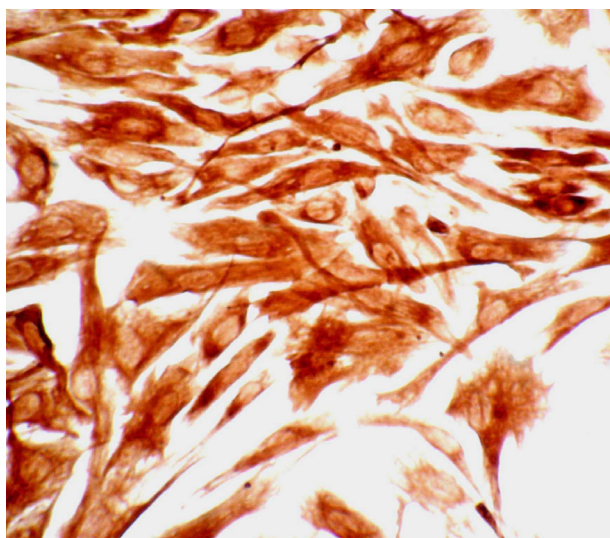


Figure 2. Immunostaining of human myoblasts for desmin expression. The cells were cultured on polylysine coated microscopic glass slides for 24 hours. Immunostaining was performed using primary mouse anti-human desmin monoclonal antibody (Sigma) and secondary rabbit anti-mouse polyclonal antibody conjugated with horseradish peroxidase enzyme. The desmin positive cells were visualized by diaminobenzidine substrate (magnification = 400x).

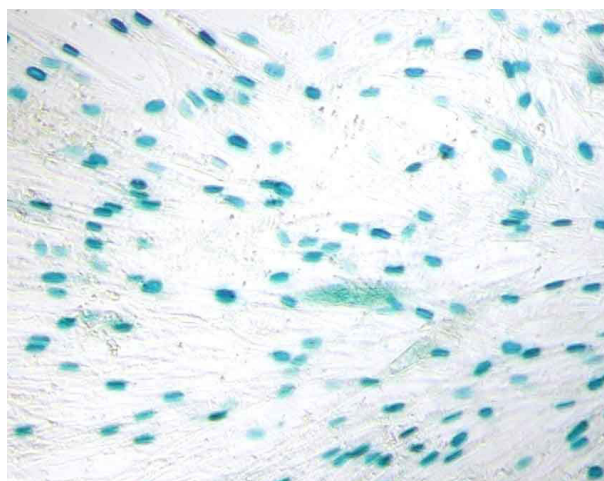


Figure 3. Histochemical staining for β -gal activity in human myoblasts in vitro. The cells were transduced with *lac-z* reporter gene using a retroviral vector construct with nuclear localization signal. The cells were stained with x-gal substrate. The figure shows green stained nuclei of the myoblasts (Magnification = 200 X 1.5x).

also raises the possibility of using non-human cells with favorable characteristics to be used in human subjects.

The in vivo behaviour of human myoblasts with respect to cellular cardiomyoplasty in heart failure patients has been less well studied due to the ethical issues difficulties of using human subjects as models. Most of the information in this regard could be based on data from the post mortem samples of the myocardium taken from the patients who died after receiving myoblast cellular grafts or from the biopsies taken from Duchene Muscular Dystrophy patients getting myoblast transplantation therapy.

We have created porcine heart models of chronic ischemia and infarction by placement of ameroid ring around the left circumflex coronary artery (LCx) at its proximal most position and by the ligation of a branch of the LCx using prolene suture respectively. Both the models were confirmed for ischemia or infarction using Tc^{99m}-MIBI rest and stress nuclear scans before transplantation of the myoblasts. The complete occlusion of the ligated coronary blood vessel was assessed by coronary angiography by the injection of the contrast agent (Hypaque-76, Nycomed Inc., New York). The injection of donor myoblast in the heart was carried out by intramyocardial injection in the free wall of the left ventricle under direct vision. Twenty injections of 0.25 ml each, containing total of 300 million cells were injected in and around the area of infarct. The control animals were injected with the same volume of injection solution, however, without cells.

Immunobiology of Myoblast Therapy

Limiting factors in the myoblast grafting for tissue repair include extensive early cell death, poor overall cell survival rate and limited translocation from the site of injection [18, 43, 46]. The initial decline in the donor cell

Human myoblasts for cardiac repair

number may be attributed to maladaptation or possible anoxia at the site of injection [10]. A second phase of cell death has also been described and ascribed to non-specific inflammatory response initiated due to tissue damage and the donor cell specific immune response.

Ideally myoblast transplantation should involve the introduction of donor cells in the host myocardium to repopulate the damaged myocardium. The cells in most cases have been obtained from autologous sources or they have been grafted in immunocompatible or the immunosuppressed hosts [19, 26]. There is increasing evidence that the immune response specific for donor myoblasts occur both in humans as well as animals [20, 45]. The human myoblasts if injected into inadequately immunosuppressed recipient may trigger immune response [19]. Without the immunosuppressive treatment, humoral reactions and antibody production are present within 2 weeks and the immune reaction leads to rejection of the graft [53]. After injection into the host, the cultured donor myoblasts pass through rapid and massive cell death phase. This is extensive by 48 hours resulting in death of up to 99% of the injected myoblasts in some cases [1]. The acute and profound cell death is too rapid to be explained alone on the basis of immunological reactions [18]. Guerrette and co-workers have shown that the initial massive cell death is caused by an inflammatory process [15]. They have demonstrated the infiltration of the injected site by polymorph nuclear cells within one hour of myoblast injection followed by infiltration by Mac1+, CD4+ CD8+ and LFA1+ [13]. Merly et al have presented evidence that the non-specific inflammatory reaction was responsible for the rapid myoblast death of up to 90% [35]. Infiltration of the injection site has been observed by neutrophils and natural killer cells within the first 5 hours of transplantation. The infiltrating cells cause cytotoxicity against the donor myoblasts. The role of host complement system in cell graft rejection has also been studied. Complement activation seems not to be directly implicated in damaging the myoblast graft but plays a secondary role to the cell death by enhancing chemotaxis of neutrophils and macrophages at the graft site [49]. Numerous studies have implicated the host immune response as the cause of cell death although the exact mechanism is not yet known.

Unlike embryonic stem cells, human myoblast behave as 'conditionally' privileged cells when they are grafted in vivo. Immunogenicity of the implanted myoblasts is expected to decline down the pathway of differentiation as myoblasts down-regulate the expression of MHC-I and MHC-II antigens. The skeletal muscle fibers do not express MHC antigens except for in the pathological conditions [39].

Strategies to Enhance Donor Cell Acceptability

Having identified acute massive cell death as a major problem, methods have been designed to modulate both humoral as well as cellular immune responses or modify the delivery strategies to enhance donor cells acceptability

by the host [14, 40, 43, 50]. The use of anti-inflammatory agents including glucocorticoids (methylprednisolone) Naproxen and Piroxicam were inadequate to effectively control the survival of the transplanted myoblasts [15]. The use of donor specific transfusion is an attractive alternative to the continued use of such drugs which have deleterious effects [3]. Treatment with anti-LFA (lymphocyte function associated antigen) antibodies reduced myoblast death to 18% [16].

Another strategy may be to use multifunctional growth factors and cytokines such as bFGF and TGF β -1 which play an integral role in immunoregulation [26]. Systemic injection of TGF β -1 or pretreatment of myoblasts with TGF β -1 results in prolonged survival of the donor myoblasts. The use of genetically modified myoblasts will release TGF β -1 locally or the other strategy may be to co-transplant myoblasts and cells carrying TGF β -1 [48]. Similarly, the myoblasts genetically modulated to produce IL-1 inhibitor may show improved survival rate post transplantation [43].

The use of immunosuppression is routinely practiced in tissue transplantation. The rejection of the donor cells has been prevented by the use of various immunosuppressants including FK-506 or its analogues or by the administration of cyclosporine-A [2, 24, 25, 27, 54]. The degree of success varies with the mode of immunosuppression and the effectiveness of immunosuppressive treatment [53]. The introduction of cyclosporine, alone or in combination with other agents, as the primary immunosuppressive agent has resulted in excellent graft cell survival [6, 55]. Our own results emphasize the importance of transient immunosuppression to achieve long term survival of the xenografted myoblasts [17]. Starting immunosuppression a few days prior to cell transplantation provides a congenial environment for donor cells to adjust to the host surroundings. In this study, 6 weeks immunosuppression was sufficient for the donor cell survival; it allowed the myoblasts to differentiate into mature fibers (Figure 4). Skeletal myoblasts express MHC antigens on their surface until they start to differentiate. During skeletal muscle development and regeneration, expression of MHC antigens is down-regulated. It is known that the differentiated muscle fibers do not express MHC-I and II [22]. The use of cyclosporine successfully prevents antibody formation against the donor myoblasts. It suppresses host adoptive immune response, thus allowing differentiation of donor myoblasts into mature muscle fibers. Secondly, it contributes towards down-regulation of MHC antigen expression.

Immunohistochemistry showed no expression of human MHC-I at 6 weeks post myoblast transplantation (Figure 5). The long-term survival of myoblasts and lack of immune response towards the xenograft as evident from the absence of immune cell infiltration at the site of the graft, is suggestive that the graft is taken as 'self' by the host immune system. The possible mechanism is sponta-

Human myoblasts for cardiac repair

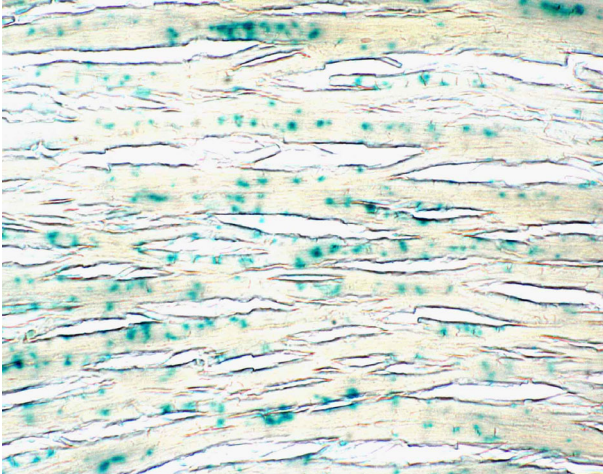


Figure 4. Lac-z expression in vivo in a porcine heart transplanted with human myoblasts at 6 weeks post human myoblast transplantation. The cells were transduced with lac-z reporter gene using retroviral construct with nuclear localization signal. The cells were transplanted by intramyocardial injections under direct vision. The green dots represent the lac-z positive donor myoblasts nuclei in the host tissue.

neous fusion between the fusion competent myoblasts and host cardiomyocytes (our unpublished data).

Histological examination of explanted porcine myocardium between 6 to 30 weeks after transplantation of myoblast showed not only myofibers of human origin, but also porcine cardiomyocytes having human myonuclei with *lac-z* gene expression. Control muscle stained sections did not show *lac-z* expression. Human myoblasts survived and integrated into the porcine ischemic myocardium, allowing concomitant cell therapy and genome therapy. Whereas new fiber formation improves heart contractility, the genetic transformation of cardiomyocytes *in vivo* to become regenerative heterokaryons through myoblast genome transfer constitutes an exciting new therapy for heart repair.

Future Perspectives

The time lag for the generation of myoblasts from the skeletal muscle biopsies and their amplification to sufficiently large number for human application is an obstacle in the clinical implementation of myoblast therapy for cardiac repair. Like the 'gene in a bottle' concept for the use of DNA as a pharmaceutical product in gene therapy, the ideal scenario for the use of myoblasts for transplantation would be to have 'ready-to-use' myoblast preparations. The transplantation of cells and organs across the species barriers is a potential field and a major effort is in progress to alleviate the concerns regarding their use in human. The potential of xenotransplantation may be exploited by genetically engineering the surface antigens on the donor cells. By genetic manipulation, they may be made friendlier for the host immune system.

Another important aspect is to adopt counter measures to meet demand for normal myoblasts. The labor inten-

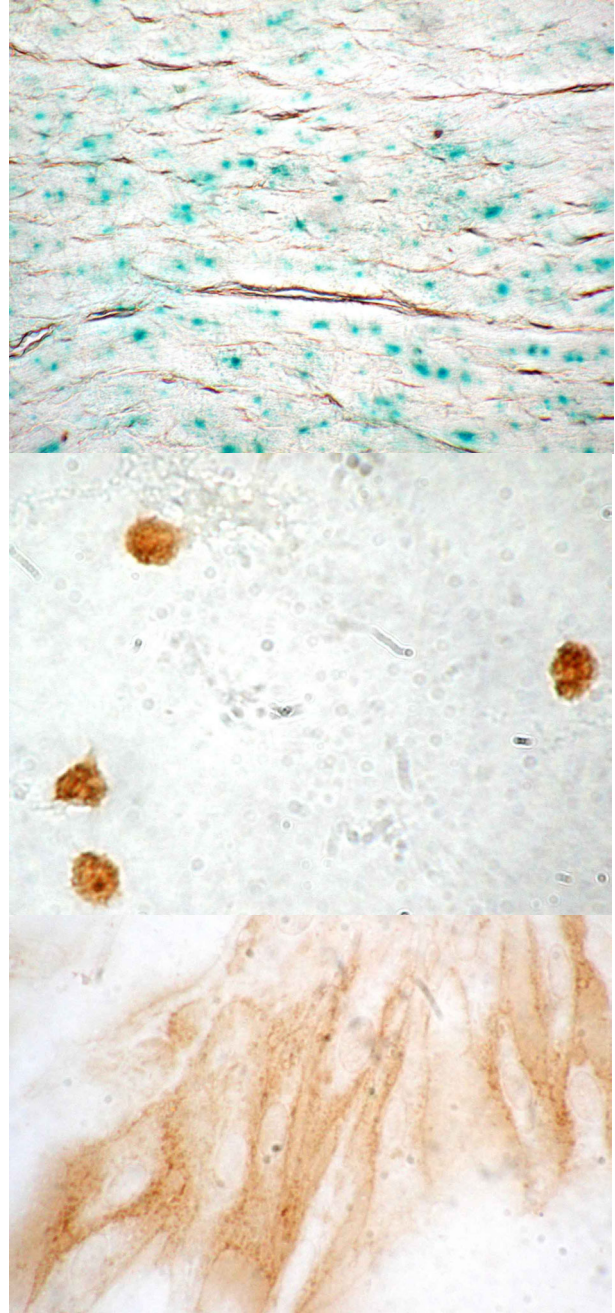


Figure 5a-c. Immunostaining of human myoblast transplanted lac-z positive porcine cardiac tissue sections (8 μ m thickness) for the expression of human major histocompatibility complex (MHC). Figures 5b and 5c represent positive controls using human blood sample smear and human myoblasts cultured in vitro on microscopic glass slides respectively and immunostained for human MHC-I expression. (Magnifications: a = 400; b & c = Oil immersion)

siveness and high cost of cell culturing, harvesting and packaging, and the fallibility of human imprecision will soon necessitate the production of automated cell processors capable of manufacturing huge quantities of viable, sterile, genetically well-defined and functionally

demonstrated myoblasts. This invention will be one of the most important offspring of modern day computer science, mechanical engineering, and cytogenetics. The intakes will be for biopsies of various human tissues. The computer may be programmed to process tissue(s), with precision controls in time, proportions of culture ingredients and apparatus maneuvers. Cell conditions can be monitored at any time during the process and flexibility is built-in to allow changes. Different protocols can be programmed into the software for culture, controlled cell fusion, harvest and package. The outputs supply injectable cells ready for cell therapy or shipment. The cell processor will be self-contained in a sterile enclosure large enough to house the hardware in which cells are cultured and manipulated.

Conclusion

The cross species survival of the graft in an immunocompetent host is not well supported by the prevailing concepts of immunotolerance. The success of clinical xenotransplantation of organs and cells depends on finding the ways of inducing tolerance across the xenogenic barriers. In view of the emerging reports of successful xenograft survival, the understanding and careful elaboration of the fundamental concepts in this regard will have far reaching implications on transplantation based treatment modalities. Unless the underlying mechanism of the grafted myoblast rejection is ascertained, it will remain a daunting task to translate myoblast transplantation into a clinical reality.

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References

- [1] Beauchamp JR, Morgan JE, Pagel CN, Partridge TA: Quantitative studies of efficacy of myoblast transplantation. *Muscle Nerve* 1994; 4 (suppl): 261.
- [2] Camirand G, Caron NJ, Asselin I, Tremblay JP: Combined immunosuppression of mycophenolate mofetil and FK506 for myoblast transplantation in mdx mice. *Transplantation* 2001; 72: 38-44.
- [3] Camirand G, Caron NJ, Turgeon NA, Rossini AA, Tremblay JP: Treatment with anti-CD154 antibody and donor-specific transfusion prevents acute rejection of myoblast transplantation. *Transplantation* 2002; 73: 453-461.
- [4] Chachques JC, Cattadori B, Herreros J, Prosper F, Trainini JC, Blanchard D, Fabiani J-N, Carpentier A: Treatment of heart failure with autologous skeletal myoblasts. *Herz* 2002; 27: 570-578.
- [5] Cooper RN, Irintchev A, Di Santo JP, Zweyer M, Morgan JE, Partridge TA, Butler-Browne GS, Mouly V, Wernig A: A new immunodeficient mouse model for human myoblast transplantation. *Hum Gene Ther* 2001; 12: 823-831.
- [6] Cosenza CA, Tusio PJ, Chapman FA, Middleton YD, Cramer DV, Wu GD, Makowka L: Prolonged xenograft survival following combination therapy with brequinar sodium and cyclosporine. *Transplant Proc* 1993; 25: 59-60.
- [7] Dib N, Diethrich EB, Campbell A, Goodwin N, Robinson B, Gilbert J, Hobohm DW, Taylor DA: Endoventricular transplantation of allogenic skeletal myoblasts in a porcine model of myocardial infarction. *J Endovasc Ther* 2002; 9: 313-319.
- [8] Dorfman J, Duong M, Zibaitis A, Pelletier MP, Shum-Tim D, Li C, Chiu RC: Myocardial tissue engineering with autologous myoblast implantation. *J Thorac Cardiovasc Surg* 1998; 116: 744-751.
- [9] Etzion S, Battler A, Barbash IM, Cagnano E, Zarin P, Granot Y, Kedes LH, Kloner RA, Leor J: Influence of embryonic cardiomyocyte transplantation on the progression of heart failure in rat model of extensive myocardial infarction. *J Mol Cell Cardiol* 2001; 33: 1321-1330.
- [10] Fan Y, Maley M, Beilharz M, Grounds M: Rapid death of injected myoblasts in myoblast transfer therapy. *Muscle & Nerve* 1996; 19: 853-860.
- [11] Gill RG, Wolf L: Immunobiology of cellular transplantation. *Cell Transplant* 1995; 4: 361-370.
- [12] Grounds MD, White JD, Rosenthal N, Bogoyevitch MA: The role of stem cells in skeletal and cardiac muscle repair. *J Histochem Cytochem* 2002; 50: 589-610.
- [13] Guerette B, Asselin I, Vilquin JT, Roy R, Tremblay JP: Lymphocyte infiltration following allo- and xenomyoblast transplantation in mdx mice. *Muscle Nerve* 1995; 18: 39-51.
- [14] Guerette B, Gingras M, Wood K, Roy R, Tremblay JP: Immunosuppression with monoclonal antibodies and CTLA4-Ig after myoblast transplantation in mice. *Transplantation* 1996; 62: 962-967.
- [15] Guerette B, Skuk D, Celestin F, Huard C, Tardif F, Asselin I, Goulet M, Roy R, Entman M, Tremblay JP: Prevention by anti-LFA-1 of acute myoblast death following transplantation. *J Immunol* 1997; 159: 2522-2531.
- [16] Guerette B, Wood K, Roy R, Tremblay JP: Efficient myoblast transplantation in mice immunosuppressed with monoclonal antibodies and CTLA4 Ig. *Transplant Proc* 1997; 29: 1932-1934.
- [17] Haider Kh H, Jiang SJ, Ye L, Teh M, Chua F, Law PK, Chua T, Sim EKW: Transient Immunosuppression is effective for xenotransplantation of human myoblasts for cardiac repair in a porcine heart model. *Circulation* 2002; 106 (19): 69.
- [18] Hodgetts SI, Beilharz MW, Scalzo AA, Grounds MD: Why do cultured transplanted myoblasts die in vivo? DNA quantification shows enhanced survival of donor male myoblasts in host mice depleted of

Human myoblasts for cardiac repair

- CD4+ and CD8+ cells or Nk1.1+ cells. *Cell Transplant* 2000; 9: 489-502.
- [19]Huard J, Roy R, Guerette B, Verreault S, Tremblay G, Tremblay JP: Human myoblast transplantation in immunodeficient and immunosuppressed mice: evidence of rejection. *Muscle & Nerve* 1994; 17: 224-234.
- [20]Irintchev A, Zweyer M, Wernig A: Cellular and molecular reactions in mouse muscles after myoblast implantation. *J Neurocytol* 1995; 24: 319-331.
- [21]Jain M, DerSimmonian H, Brenner DA, Ngoy S, Teller MA, Edge ASB, Zawadzka A, Wetzel K, Sawyer DB, Colucci WS, Apstein CS, Liao R: Cell Therapy attenuates deleterious ventricular remodeling and improves cardiac performance after myocardial infarction. *Circulation* 2001; 103: 1920-1927.
- [22]Karpati G, Pouliot Y, Carpenter S: Expression of immunoreactive major histocompatibility complex products in human skeletal muscles. *Ann Neurol* 1988; 23: 64-72.
- [23]Kessler PD, Byrne BJ: Myoblast cell grafting in to heart muscle: Cellular Biology and potential applications. *Ann Rev Physiol* 1999; 61: 219-242.
- [24]Kinoshita I, Vilquin JT, Guerette B, Asselin I, Roy R, Lille S, Tremblay JP: Immunosuppression with FK 506 ensures good success of myoblast transplantation in MDX mice. *Transplant Proc* 1994; 26: 3518.
- [25]Kinoshita I, Vilquin JT, Guerette B, Asselin I, Roy R, Tremblay JP: Very efficient myoblast allotransplantation in mice under FK506 immunosuppression. *Muscle & Nerve* 1994; 17: 1407-1415.
- [26]Kinoshita I, Vilquin JT, Gravel C, Roy R, Tremblay JP: Myoblast allotransplantation in primates. *Muscle & Nerve* 1995; 18: 1217-1218.
- [27]Kinoshita I, Roy R, Dugre FJ, Gravel C, Roy B, Goulet M, Asselin I, Tremblay JP: Myoblast transplantation in monkeys: control of immune response by FK506. *J Neuropathol Exp Neurol* 1996; 55: 687-697.
- [28]Law PK: Compositions for and methods of treating muscle degeneration and weakness, U.S. Patent No. 5,130,141, issued July 14, 1992.
- [29]Law PK, "Myoblast therapy for mammalian diseases", Singapore Patent No. 34490 (WO 96/18303), issued August 22, 2000.
- [30]Leichty KW, Mackenzie TC, Shaaban AF, Radu A, Moseley AB, Deans R, Marshak DR, Flake AW: Human mesenchymal stem cells engraft and demonstrate site specific differentiation after in utero transplantation in sheep. *Nat Med* 2000; 6: 1282-1286.
- [31]Li RK, Weisel RD, Mickel DAG, Jia ZQ, Kim EJ, Sakai T, Tomita S, Schwartz L, Iwanochko M, Husain M, Cushman RJ, Burns RJ, Yau TM: Autologous porcine heart cell transplantation improved heart function after a myocardial infarction. *J Thorac Cardiovasc Surg* 2000; 119: 62-68.
- [32]Menasche P, Hagege AA, Scorsin M, Pouzet B, Desnos M, Duboc D, Schwartz K, Vilquin JM, Marolleau JP: Myoblast transplantation for heart failure. *Lancet* 2001; 357: 279-280.
- [33]Menache P, Vilquin J-T, Densos M, Duboc D, Hagege AA, Pouzet B, Scorsin M, Schwartz K: Early results of autologous skeletal myoblast transplantation in patients with severe ischemic heart disease. *Circulation* 2001; 104 (Suppl-II): II-598.
- [34]Menasche P: Cell transplantation for the treatment of heart failure. *Semin Thorac Cardiovasc Surg* 2002; 14: 157-166.
- [35]Merly F, Huard C, Asselin I, Robbins PD, Tremblay JP: Anti-inflammatory effect of transforming growth factor- β 1 in myoblast transplantation. *Transplant* 1998; 65: 793-799.
- [36]Min J-Y, Sullivan MF, Yang Y, Zhang J-P, Converso KL, Morgan JP, Xiao Y-F: Significant improvement of heart function by co-transplantation of human mesenchymal stem cells and fetal cardiomyocytes in post infarcted pigs. *Ann Thorac Surg* 2002; 74: 1568-1575.
- [37]Min J-Y, Yang Y, Converso KL, Liu L, Huang Q, Morgan JP, Xiao Y-F: Transplantation of embryonic stem cells improves cardiac function in post-infarcted rats. *J Appl Physiol* 2002; 92: 288-296.
- [38]Orlic D, Kajstura J, Chimenti S, Limana F, Jokaniuk I, Quain F, Nodal-Ginard B, Bodine DM, Leri A, Anversa P: Mobilized bone marrow cells repair the infarcted heart improving function and survival. *Proc Natl Acad Sci USA* 2001; 98: 10344-10349.
- [39]Pavlati GK: Regulation of class I MHC expression in skeletal muscle: deleterious effect of aberrant expression on myogenesis. *J Neuroimmunol* 2002; 125: 42-50.
- [40]Peirone M, Ross CJ, Hortelano G, Brash JL, Chang PL: Encapsulation of various recombinant mammalian cell types in different alginate microcapsules. *J Biomed Mater Res* 1998; 42: 587-596.
- [41]Pouzet B, Vilquin JM, Hagege AA, Scorsin M, Emmanuel M, Fiszman M, Schwartz K, Menache P: Intramyocardial transplantation of autologous myoblasts: can tissue processing be optimized? *Circulation* 2000; 102 (Suppl III): III-210-215, 2000.
- [42]Pouzet B, Vilquin JT, Hagege A, Scorsin M, Mesas E, Fiszman M, Schwartz K, Menasche P: Factors effecting functional outcome of autologous skeletal myoblast transplantation. *Ann Thorac Surg* 2001; 71: 844-511.
- [43]Qu Z, Balkir L, Van Deutekom JCT, Robbins PD, Pruchnic R: Development of approaches to improve cell survival. *J Cell Biol* 1998; 142: 1257-1267.
- [44]Rajnoch C, Chachques JC, Berrebi A, Bruneval P, Benoit MO, Carpentier A: Cellular therapy reverses myocardial dysfunction. *J Thorac Cardiovasc Surg* 2001; 121: 871-878.
- [45]Rando TA, Blau HM: Primary mouse myoblast purification, characterization, and transplantation for cell-mediated gene therapy. *J Cell Biol* 1994; 125: 1275-1287.

Human myoblasts for cardiac repair

- [46]Rando TA, Pavlath GK, Blau HM: The fate of myoblasts following transplantation into mature muscle. *Exp Cell Res* 1995; 220: 383-389.
- [47]Saito T, Kuang J-Q, Bittira B, Al-Khalidi A, Chiu R C-J: Xenotransplant cardiac chimera: immune tolerance of adult stem cells. *Ann Thorac Surg* 2002; 74: 719-724.
- [48]Schneider BL, Peduto G, Aebischer P: A self-immunomodulating myoblast cell line for erythropoietin delivery. *Gene Ther* 2001; 8: 58-66.
- [49]Skuk D, Tremblay JP: Complement deposition and cell death after myoblast transplantation. *Cell Transplant* 1998; 7: 727-734.
- [50]Skuk D, Goulet M, Roy B, Tremblay JP: Efficacy of myoblast transplantation in nonhuman primates following simple intramuscular cell injections: toward defining strategies applicable to humans. *Exp Neurol* 2002; 175: 112-126.
- [51]Sun H, Wakizaka Y, Rao AS, Pan F, Madariaga J, Park IY, Celli S, Fung JJ, Starzl TE, Valdivia LA: Use of MHC class I or II "knock out" mice to delineate the role of these molecules in acceptance/rejection of xenografts. *Transplant Proc* 1996; 28: 732.
- [52]Taylor DA, Atkin BZ, Hungspecugs P, Jones TR, Reedy MC, Hutcheson KA, Glower DD, Kraus WE: Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. *Nat Med* 1998; 4: 929-933.
- [53]Vilquin JT, Asselin I, Guerette B, Kinoshita I, Lille S, Roy R, Tremblay JP: Myoblast allotransplantation in mice: degree of success varies depending on the efficacy of various immunosuppressive treatments. *Transplant Proc* 1994; 26: 3372-3373.
- [54]Vilquin JT, Kinoshita I, Roy R, Tremblay JP: Cyclophosphamide immunosuppression does not permit successful myoblast allotransplantation in mouse. *Neuromuscul Disord* 1995; 5: 511-517.
- [55]Wu GS, Korsgren O, Tibell A: Cyclosporine induces long-term xenograft survival in the mouse-to-C6-deficient rat heart transplantation model. *Transplant Proc* 2000; 32: 1015.
- [56]Zammit PS, Beauchamp JR: The skeletal muscle satellite cell: stem cell or son of stem cell? *Differentiation* 2001; 68: 193-204.