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Therapeutic angiomyogenesis using human non-viral transduced VEGF$_{165}$-myoblasts*

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ABSTRACT

This article reviews the scientific development of angiomyogenesis using VEGF$_{165}$-myoblasts, a patented biotechnology platform in regenerative medicine associated with Human Myoblast Genome Therapy (HMGT), also known as Myoblast Transfer Therapy (MTT). VEGF$_{165}$-myoblasts are the leading biologics for angiomyogenesis. This review also compares the safety and efficacy of VEGF$_{165}$-myoblasts transduced using adenoviral vectors, nanoparticles or liposomes, in anticipation of their application in clinical trials in the near future. VEGF$_{165}$-myoblasts are differentiated myogenic cells capable of extensive division, natural cell fusion, nucleus transfer, cell therapy and genome therapy. Following transplantation they survive, develop and function to revitalize degenerative myocardium in heart failure and ischemic cardiomyopathy animal studies. VEGF$_{165}$-myoblasts are second generation products of HMGT/MTT which replenishes live cells and genetically repairs degenerating myofibers in Type II diabetes, muscular dystrophies, aging dysfunction and disfigurement. Myoblasts have also been used to enhance skin and muscle appearance in cosmetology. We envision that VEGF$_{165}$-myoblasts will provide better outcome than their non-tranduced counterparts. Myoblasts are not stem cells. Their competitive advantages over stem cells are presented.

Keywords: Angiomyogenesis; Cell Therapy; Gene Therapy; VEGF$_{165}$-Myoblasts; Muscular Dystrophies; Heart Failure; Ischemic Cardiomyopathy; Type II diabetes; Anti-Aging Cosmetics; Sexual Impotency; Baldness; Stem Cells

1. INTRODUCTION

The human body consists of living cells supplied 100% with capillaries. More than 55% by volume of these cells are myogenic. Muscle degeneration is the common pathway of many fatal and debilitating diseases such as muscular dystrophies, heart failure, ischemic cardiomyopathy, Type II diabetes and aging disfigurement. Therapeutic angiomyogenesis, the concomitant regeneration of muscles and capillaries using biologics is the leading approach to combat these most eminent killers of mankind. This has already been achieved in animals with the unique platform technology of VEGF$_{165}$-myoblast transfer.

2. BIOLOGICS: CELLULAR VS. MOLECULAR

The cell is the origin of all life. Contained within its nucleus are more than 30,000 genes that determine cell normality and cell characteristics [1]. The genes are composed of deoxyribonucleic acid (DNA) that is spatially and temporally switched on and off during development to produce more than 100,000 different transcripts of ribonucleic acid (RNA). The transcriptional events occur inside the nucleus and require the nuclear matrix and/or the chromatin to operate efficiently. These regulatory events are poorly understood but invariably involve polygenic interactions.

Whereas the basic sequence of the human genome has been determined, exactly how the genome functions will take many decades of further research. Scientists do not know the spatial and temporal interactions of the RNA transcripts and know little of their modes of action. Numerous methods have yet to be developed to determine the diverse functions of some 30,000 genes and more techniques have to be refined to effect gene regulation

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and expression. It is through this knowledge that pharmacogenetics may one day provide rational approaches in therapeutics. Today, no genetic treatment of human disease has been developed based on the Human Genome Project. The analysis of DNA/RNA variations and gene expression are used mainly in diagnostics, while gene therapy success through single gene manipulation has been rare.

3. HMGT/MTT: A COMBINED CELL THERAPY AND GENOME THERAPY

An alternative perspective is that a genetically abnormal cell degenerates due to the lack of the normal genome. In hereditary degenerative diseases such as muscular dystrophies, the much-needed normal genome can be incorporated into the dystrophic muscle fibers. This is achieved by taking a muscle biopsy from normal male donors, culturing pure mono-lineaged myogenic cells called "myoblasts" and injecting the normal myoblasts into dystrophic muscles. This cell transplant procedure is called myoblast transfer therapy (MTT) or human myoblast genome therapy (HMGT). Myoblast transfer is a treatment of important potential value [2].

Through natural cell fusion, which is inherent in myogenesis and muscle regeneration, the donor myoblasts insert their normal nuclei that contain the human genome into the dystrophic muscle fibers, forming multinucleated heterokaryons to effect genetic complementation repair [3]. The donor nuclei operate to transcribe the missing RNA. Only transfer of the normal nuclei, carrying the genomic software and the chromosomal hardware, will allow the orderly provision of various co-factors necessary for the regulation and the expression of the transgene [4].

Natural transcription of the normal genome within the donor nuclei following MTT ensures orderly replacement of any protein deficiency resulting from single gene defect such as dystrophin for Duchenne muscular dystrophy (DMD), or from haphazard polygenic interactions such as GLUT4/IRAP for Type II diabetes. This differs significantly from single gene transduction, effected through viral or non-viral vectors, in that the transgene may find no transcriptional factors/co-factors in the adult environment for its regulation and expression. Many of these co-factors are the products of other genes that are only operative in early development. In addition, myoblasts are normal human cells and do not have complications of the Gene Therapy Medicinal Products (**Table 1**).

HMGT/MTT is a patented platform technology of cell transplantation, nuclear transfer, genome therapy and tissue engineering. It is the only human genome therapy in existence, and will remain so until another modality is discovered to deliver the human genome into the defective cells of a genetically ill patient. Myoblast is the only somatic cell type that has the ability of natural cell fusion. MTT is uniquely suited to treat hereditary muscle degeneration and weakness through nuclear transfer or genome transfer. In addition, when donor myoblasts fuse among themselves after MTT, they form new muscle fibers to repopulate the degenerative organ, depositing contractile filaments to augment its function. Thus, as a cell therapy, MTT find application not only to all forms of skeletal muscle degeneration, but to heart muscle degeneration, body-building, anti-ageing and soft tissue enhancement [5]. MTT replenishes live cells through cell therapy, and repairs degenerating cells through genome therapy.

The platform biotechnology of HMGT/MTT using transduced or non-transduced myoblasts to treat a host of human diseases and conditions has been the subject of many patents awarded worldwide [6-25].

4. HMGT/MTT FOR MUSCULAR DYSTROPIES

First conducted in February 1990 and published on 14 July 1990, MTT is the world's first human gene therapy [3]. This contention is not without contest [26-29], and only the record can set it straight for such an important claim.

HMGT/ MTT procedures have been conducted on patients suffering from Duchenne, Becker or Limb-Girdle muscular dystrophies. Beginning with 8 million myoblasts into a small foot muscle, Law's team proceeded to test 5 billion cells into 22 leg muscles, 25 billion cells into 64 body muscles, and then 50 billion cells into 82 muscles. With 280 procedures having been conducted to date, the complete safety of the HMGT/MTT procedure has been proven [3,5,30-35].

There have been no adverse reactions or side effects. There has not been a single death or coma or failure in heart, lung, kidney or liver function related to the MTT procedures. Expected adverse reactions include mild fever (<101˚F), pain, nausea and/or erythema lasting up to three

Table 1. Complications of viral and non-viral vectors and plasmids.

Viral Vectors	Non-Viral	Plasmids
Immunogenicity	Toxicity	Immunogenicity
Inflammation		Random integration
Tumorigenicity		Toxicity
Random integration		
Virulence		
Replication		
Latency/Reactivation		

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to prevent rejection of the foreign myoblasts because days. Two months of immunosuppression is sufficient mature muscle fibers do not express MHC-1 surface antigens.

Therapeutic gene expression of dystrophin is efficient and stable, lasting up to six years after MTT [36]. Phase II/III clinical trials had provided significant safety and efficacy data (**Table 2**) that the US Food and Drug Administration (FDA) approved direct cost recovery.

5. HEART CELL THERAPY (HCT)

Heart muscle degeneration is the leading cause of debilitation and death in humans. It results in loss of live cardiomyocytes, contractile filaments, contractility, heart function and healthy circulation. The damaged heart responds by cell division of cardiomyocytes. Cardiomyocytes do not multiply significantly because the human telomeric DNA repeats in these terminally differentiated cells are minimal. Without significant mitotic activity, surviving cardiomyocytes cannot provide enough new cells to deposit the contractile filaments necessary to sustain normal heart contractility.

Bioengineering the regenerative heart has been shown to provide a potential treatment for cardiovascular diseases. Through endomyocardial injections of cultured skeletal myoblasts, the latter spontaneously transfer their nuclei into cardiomyocytes to impart myogenic regeneration. Injected myoblasts trans-differentiate to become cardiomyocytes. Donor myoblasts also fuse among themselves to form new myofibers, depositing contractile filaments to improve heart contractility. These myofibers contain satellite cells with regenerative vigor to combat heart muscle degeneration.

Three myogenesis mechanisms were elucidated as proof of concept with 50 human/porcine xenografts using cyclosporine as immunosuppressant [37-39]. Some myoblasts trans-differentiated to become cardiomyocytes. Others transferred their nuclei into host cardiomyocytes through natural cell fusion. As yet others formed skeletal myofibers with satellite cells. *De novo* production of contractile filaments augmented heart contractility [40, 41]. Whereas the newly formed myofibers harbor satellite cells and impart regenerative capacity to the heart muscle, the genetic transformation of cardiomyocytes *in vivo* to become regenerative heterokaryons through myoblast genome transfer [37] constitutes the ultimate heart repair. The regenerative heart [38] also contains trans-differentiated cardiomyocytes of myoblastic origin. In all three scenarios, new contractile filaments are deposited to improve heart contractility. This latter can be translated into the improvement in the quality of life of heart patients and in the prevention of heart attacks.

The *first* human myoblast transfer into the heart revealed that it was safe to administer one billion myoblasts at 100×10^6/ml through the Myostar catheter of the NOGA system (Biosense Webster Inc.) using 20 injections at different locations inside the left ventricle of a swine [42,43]. It was determined that 0.3 ml to 0.5 ml would be the optimal volume per injection. EKG was normal throughout the study without arrhythmia.

Table 2. Efficacy demonstrated in myoblast—injected DMD muscles in US-FDA approved 50-B MTT Phase II/III clinical trials.

1	Dystrophin
2	70% more myofibers and histological improvement
3	123% increase in contractile force at 18 mo post
4	39% decrease in CPK
5	19% increase in forced vital capacity at 9 mo post
6	Clinical improvement in 75% of all subjects

Heart Cell Therapy (HCT) [44], as this is called, is administered with the vision that the myoblasts will survive, develop and function as aliens in the heart, and their nuclei as aliens within cardiomyocytes and myofibers. The myocardial aliens are newly formed skeletal myofibers that contribute to cardiac output through production of contractile filaments. They are donor in origin and as skeletal myofibers will have satellite cells and regenerative capability. The cardiomyocyte aliens are donor myoblast nuclei carrying chromosomes with long telomeric DNA subunits that are essential for mitosis. Upon injury of the trans-differentiated or heterokaryotic cardiomyocyte, the myoblast regenerative genome will be activated to produce foreign contractile filaments such as myosin and actin.

There was a transient elevation of the porcine anti-human-myoblast antibodies at one week after the xenograft [45-47]. The antibody level subsided at the second week after HMGT, indicating that no more than two weeks of cyclosporine immunosuppression would be necessary for human/pig xenografts or for human allografts.

Myoblasts can be transduced to secrete $VEGF_{165}$ or other angiogenic factors to promote survival, development and functioning after HCT [7]. In addition, the use of controlled cell-fusion technologies enables cell fusion between myoblasts and cardiomyocytes, producing heterokaryotic cardiomyocytes that are capable of extensive mitosis [8,12].

When compared with a heart transplant, HCT eliminates the use of lifelong immune-suppressants, which is the major cause of infection and death of heart transplant patients. HCT is much less invasive, and tissue availability is not an issue. At a fraction of the cost of a heart transplant, it also promises a reduction in health costs.

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6. THERAPEUTIC ANGIOMYOGENESIS

Therapeutic angiogenesis alleviates tissue ischemia through induction of the intrinsic process of angiogenesis [48]. Vascular endothelial growth factor 165 ($VEGF_{165}$) is the major angiogenic factor involved in physiological as well as pathological angiogenesis [49]. The neovascularization is mainly achieved by endothelial cell proliferation triggered through $VEGF_{165}$ receptors, especially the $VEGF_{165}$ receptor-2 [50]. Angiogenesis occurs by sprouting of the former vasculature using previously differentiated cells [51]. Therapeutic angiogenesis exploits the natural process for enhanced neovascularization [52]. Transplantation of genetically modified myoblasts provides a reservoir to produce biologically active angiogenic factors in a localized and sustained pattern. The technology not only induces new capillary formation, but also allows the transduced myoblasts to differentiate into cardiomyocytes to restore injured myocardium, a process called angiomyogenesis [39,47,48].

In the angiomyogenesis study, human myoblasts were transduced with viral vectors, nano-particles or CD liposomes carrying human $VEGF_{165}$ genes. [47,48,53-55]. The cells were characterized for $VEGF_{165}$ transduction and expression efficiency by immunostaining, enzyme-linked immunosorbent assay (ELISA), immunoblotting and RT-PCR. A porcine heart model of infarction was created in female swine by left circumflex artery ligation. The animals were grouped either as control or myoblast-implanted. Angiography was performed to ensure complete occlusion of the blood vessel. Infarction was confirmed with MIBI-Tc99mTc-tetrofosmin SPECT scanning. Four weeks later, 5ml basal DMEM without or with 3×10^8 human myoblasts carrying $VEGF_{165}$ and Lac-Z genes were implanted into the infarcted areas of the porcine left ventricles. The animals were maintained on cyclosporine (5 mg/kg body weight) for six weeks post-operatively. Hearts were then explanted and processed for immunocytochemical studies. Increased blood vessel density and heart function were the result of these transduced myoblasts treatment [39,48,53-55].

7. SECOND GENERATION PRODUCTS

Human myoblasts transduced with $VEGF_{165}$ using Polyethylenimine-25 nanoparticles or CD liposomes are second generation products for heart angiomyogenesis (**Table 3**) [54,55]. They are promising biologics in treating ischemic cardiomyopathy. Implantation of these myoblasts is safe and efficacious in inducing angiomyogenesis and recovery of left ventricular function of infarcted rat hearts. Approximately five times more capillaries blood capillaries and muscle were found in the $VEGF_{165}$-myoblast injected myocardia as compared to the controls injected with carrier solution (**Figure 1**).

Table 3. Human VEGF Transduction Using Adenovirus, Nano-Particles, and Liposome.

Viral	Non-Viral	
Ad-$VEGF_{165}$-myoblasts	PEI-$VEGF_{165}$-myoblasts	CD-$VEGF_{165}$-myoblasts
Transduction efficiency		
95%	11%	8%
Peak VEGF		
37 ng/mL on 8th day	20.2 ng/mL on 2nd day	13.1 ng/mL on 2nd day
Decreases to		
20 ng/mL on 30th day	47 ng/ml on 18th day	41.7 ng/ml on 18th day

2.78 + 0.2 ng/ml VEGF protein efficiently stimulated neovascularization [66].

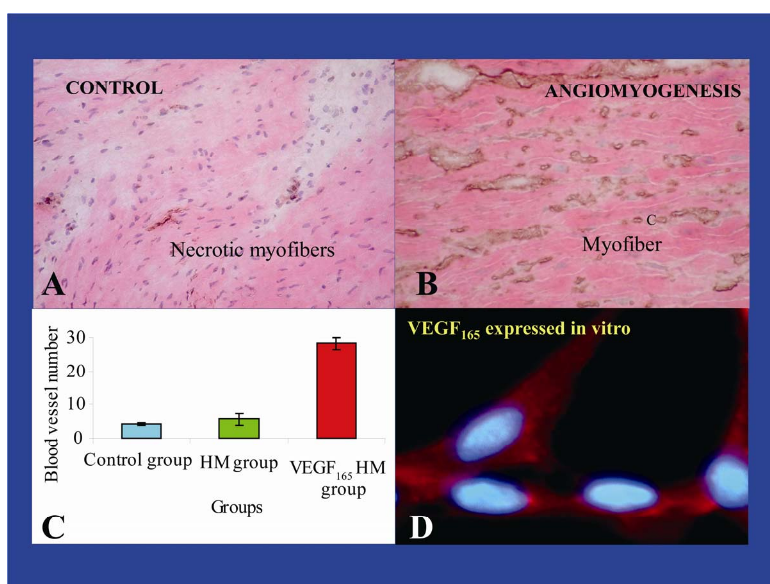

Figure 1. $VEGF_{165}$-myoblasts produced 5 times more capillaries and myofibers in infarcted pig myocardium. C, capillaries; HM, human myoblasts.

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8. AD-VEGF$_{165}$-MYOBLASTS

Various viral vectors have demonstrated high transduction efficiency of therapeutic genes into myoblasts [53,55-58]. Nevertheless, their use has demerits including immunogenicity and oncogenic potential which severely hinder their clinical application [59]. Human clinical trials have shown that viral vector based delivery of genes caused inflammatory reactions, formation of anti-adenoviral antibody, transient fever, and increase of liver transaminase [60-62].

As determined by ELISA, non-transduced myoblasts secreted VEGF$_{165}$ *in vitro* (300 ± 50 pg/mL) that is enhanced many folds (37 ± 3 ng/mL) in VEGF$_{165}$-myoblasts transduced with a replication incompetent adenoviral vector. Immunostaining showed >95% VEGF$_{165}$ positive myoblasts following transduction. Concentration of VEGF$_{165}$ released in the culture medium peaked (37 ± 3 ng/mL) at 8 days post-transduction. Cell proliferation assay on human umbilical vein endothelial cells using supernatant from VEGF$_{165}$ transduced myoblasts revealed extensive proliferation of cells which was suppressed in the presence of anti-human VEGF$_{165}$ antibody in culture medium and was further confirmed by thymidine incorporation assay [53,54].

9. PEI-VEGF$_{165}$ MYOBLASTS

Non-viral vector gene delivery approach provides a safer alternative to overcome the untoward effects of viral vectors. Use of plasmid DNA either alone or in association with cationic liposomes/polymers were assessed with encouraging results [63,64]. Similarly, the use of polymer based nanoparticles conferred several advantages including ease of preparation, purification and chemical modification as well as their enormous stability [64,65]. Polyethylenimine (PEI) has been widely used for nonviral transduction of cells [65]. PEI is cationic in nature and has strong DNA compaction capacity, effective DNA protection and with an intrinsic endosomolytic activity [65]. All these properties of PEI contribute to its transfection efficacy. PEI (molecular weight 25 kDa: PEI-25) nanoparticles were designed and transduction conditions optimized to transduce the VEGF$_{165}$ gene into human myoblasts with minimum cytotoxic effects.

The feasibility and efficacy of implanting PEI-VEGF$_{165}$ myoblasts for angiomyogenic treatment of rat infarcted myocardium were confirmed. Experimental design consisted of rat heart model of acute myocardial infarction receiving injections in group-1/DMEM, group-2/non-transduced myoblasts and group-3/PEI-VEGF$_{165}$-myoblasts. Rats were immuno-suppressed with cyclosporine injection from 3 days before and until 4 weeks after cell transplantation.

Based on optimized transfection conditions, PEI-VEGF$_{165}$-myoblasts expressed VEGF$_{165}$ for 18 days with >90% cell viability *in vitro*. Apoptotic index was reduced in group-2 and group-3 as compared with group-1. Blood vessel density (×400) by immunostaining for PECAM-1 in group-3 was significantly higher (P = 0.043 for both) as compared with group-1 and group-2 at 4 weeks. Regional blood flow (ml/min/g) in the left ventricular anterior wall was higher in group-3 (P = 0.043 for both) as compared with group-1 and group-2. Improved ejection fraction was achieved in group-3 (58.44 ± 4.92%) as compared with group-1 (P = 0.004). It was concluded that PEI-VEGF$_{165}$ myoblasts served as an efficient alternative for angiomyogenesis in cardiac repair. We anticipate that the use of PEI nanoparticles for VEGF$_{165}$ transduction of human myoblasts is a safe and efficient approach for repair of the infarcted heart. Nanoparticle gene transduction will envision a new approach for gene therapy in human cardiovascular research [54].

10. CD-VEGF$_{165}$-MYOBLASTS

CD lipoplexes were constructed using cholesterol (Chol) + DOTAP liposome (CD liposome). The efficiency of CD lipoplexes for VEGF$_{165}$ gene transfer with human myoblasts was characterized using plasmid carrying enhanced green fluorescent protein (pEGFP). Flow cytometry revealed transduction of 7.99% myoblasts with pEGFP. Human myoblasts were then transduced with CD lipoplexes carrying plasmid-VEGF$_{165}$. Based on optimized transduction condition *in vitro*, CD-VEGF$_{165}$-myoblasts expressed VEGF$_{165}$ up to day-18 (1.7± 0.1 ng/ml), peaking at day-2 (13.1 ± 0.52 ng/ml) with >85% cell viability [55].

Experimental design consisted of rat heart model of acute myocardial infarction receiving injections in group-1/DMEM, group-2/non-transduced myoblasts and group-3/CD-VEGF$_{165}$-myoblasts. Rats were immuno-suppressed with daily cyclosporine injections beginning at 3 days before and ending at 4 weeks after CD-VEGF$_{165}$-myoblast transfer. Animal studies revealed reduced apoptosis and improved increased blood flow in group-3 as compared to group-1 and 2. Ejection fraction was best recovered in group-3 animals [55].

Although VEGF$_{165}$ gene transduction efficiency was low with CD-VEGF$_{165}$-myoblasts, their gene expression efficiency was sufficient to induce neovascularization, improving blood flow and contractility in infracted rat myocardium. Even a low liposome transduction efficiency with minimal therapeutic protein production can be compensated with a large quantity of myoblast transferred. A substantial amount of the therapeutic gene can be harbored. The transduced muscle acts as a stable bioreactor to deliver a long-term supply of therapeutic protein at basal level.

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11. MYOBLASTS ARE NOT STEM CELLS

By definition, a stem cell is a non-differentiated cell that can differentiate into at least two different cell types. Stem cells are pluripotent and can differentiate into multiple lineages of cell types such as cardiomyocytes, myoblasts, fibroblasts, adipocytes, osteoblasts, and chondrocytes. Myoblasts are not stem cells because they are already differentiated and can only develop to become one cell type which is muscle.

Today, the field of cell therapy is blemished by controversial stem-cell treatments, whereas gene therapy is tainted by mishaps of 'viral vector' technology. HMGT/MTT does not involve the use of controversial stem cells or the use of dangerous viral agents that have been the cause of death in various clinical trials [59-61]. MTT/HMGT has been proven safe on approximately 280 human muscular dystrophy procedures and 300 human heart procedures since year 1990 [67]. There are much competitive advantages of HMGT/MTT over stem cell therapy for somatic tissues (**Table 4**).

Making patient safety the centerpiece of medical liability reform in USA [68], one must realize that the major deficiency of the stem cell technology is scientific. Scientists do not know the transcriptional pathways that trigger stem cells to develop along specific lineages, e.g., to differentiate only into heart muscle cells and not into other cell types. Such knowledge is not likely to be available in the near future. Until then, stem-cell transplants into the heart for example may result in bony, cartilageous, fatty and fibrotic elements that are detrimental to heart function [67].

No effect of intracoronary injection of autologous mononuclear bone marrow stem cells on global left ventricular function was found [69]. Whereas other pilot trials suggested that the intracoronary infusion of autologous progenitor cells might have improved left ventricular function after acute myocardial infarction, the statistics of these studies and thus the study conclusions could not hold because the standard deviations were greater than the means of the left ventricular ejection fractions reported [70,71]. There was no evidence of donor cell engraftment. One must critically question how the simple act of intracoronary infusion of cells could lead to engraftment.

Table 4. The competitive advantages of myoblasts over stem cells.

Stem Cells	Myoblasts
Differentiate into undesirable tissues	Develop into muscle only
Tumorgenic	Non-tumorgenic
Difficult to purify	Can be 100% purified
Difficult to mass produced	Can be mass-produced

12. VEGF$_{165}$-MYOBLAST TARGET SPECTRUM

It is anticipated that therapeutic angiomyogenesis using PEI-VEGF$_{165}$-myoblasts or CD-VEGF$_{165}$-myoblasts will find applications in the development of treatments for human heart failure, myocardial infarction in ischemic cardiomyopathy, ischemic limb and diabetic cardiomyopathy in Type II diabetes, various forms of muscular dystrophies and male/female impotency. In addition, newly regenerated capillaries and muscle cells will provide booster tissues for the construction of thicker and redder lips, prominent noses, cheeks and jaws, pink face, and revitalized hair base. Layers of myogenic cells densely populated with capillaries will provide a fertile ground to seed new hair follicle cells on the bald head or other parts of the body to grow hairs of desirable color, density and consistency.

13. CONCLUSION

Animal experimental data have culminated that pure VEGF$_{165}$-myoblasts, when injected intramyocardially, are potential therapeutic transgene vehicles for concomitant angiogenesis and myogenesis to treat heart failure and ischemic cardiomyopathy. Therapeutic angiomyogenesis has potential application to a host of fatal and debilitating diseases and conditions. In anticipation of its transitional application into clinical trials in the near future, we envision that non-viral transduced VEGF$_{165}$-myoblasts will provide better outcome than their non-tranduced counterparts.

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ABBREVIATIONS

Ad-VEGF165 = Adenoviral transduced plasmid of Vascular Endothelial Growth Factor 165 (human)
BMD = Becker Muscular Dystrophy
CABG = Coronary Artery Bypass Grafting
CD-VEGF$_{165}$ = CD liposome transduced plasmid of Vascular Endothelial Growth Factor 165 (human)
DMEM = Dulbecco's Modified Eagles Medium
DNA = Deoxyribonucleic Acid
ELISA = Enzyme-Linked Immunosorbent Assay
FDA = US Food and Drug Administration
GLUT4 /IRAP = Glucose Transporter 4/ Insulin-regulated Aminopeptidase
HCT = Heart Cell Therapy
HMGT = Human Myoblast Genome Therapy
LVEF = Left Ventricular Ejection Fraction
MHC-1 = Major Histocompatibility Class-1
MIBI-Tc99m = Technetium (99mTc) Sestamibi
MTT = Myoblast Transfer Therapy
PEI-VEGF$_{165}$ = Polyethylenimine-25 nanoparticle transduced plasmid of Vascular Endothelial Growth Factor 165 (human)
RT-PCR = Reverse Transcription Polymerase Chain Reaction
SPECT = Single-Photon Emission Computed Tomography
VEGF165 = Vascular Endothelial Growth Factor 165 (human)

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