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Genetic Cell Therapy in Anti-Aging Regenerative Cosmetology

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Abstract

As post-WWII baby boomer approaching age 80, Anti-Aging Regenerative Cosmetology (AARC) has been developed and patented for beautifying and strengthening the human body using live cells; to enhance the appearance and function of various bodily parts to provide health and aestheticism of human being throughout life. It is a combined cosmetic and preventive medicine to intervene with and to correct the undesirable phenotypic expression of aging. The intrinsic properties of myoblasts and foreskin fibroblasts in development and regeneration are harnessed to formulate a genetic cell therapy program which is safe and efficacious as previously been tested in FDA Phase III clinical trials. Myoblasts are selected for strength development and foreskin fibroblasts for tenacity and smooth-to-the-touch. Both cell types are highly mitotic and non-carcinogenic. In additional to providing large quantities of nuclei as regenerative gene medicine, and of mitochondria as energy generators, myoblasts secret tumor necrosis factor alpha (TNF-α) for skin whitening and melanoma prevention. Myoblasts, because of their small size, spindle shape, and resilience, grow readily on collagen and laminin within wrinkles of skin surfaces, thus enhancing the color, luster, and texture of the skin "plated" with them. Alternatively, they can be injected subcutaneously as cell filler to reduce wrinkles. Intramuscular injection of myoblasts can augment the size, shape, consistency, tone, and strength of muscle groups, improving the lines, contours, and vitality to sculpt a youthful appearance. By improving cell genetics and organ functions, the program holds promise to sustain the human subject in good health and appearance, with a good quality of life and life prolongation.

Keywords

Anti-Aging, Genetic Cell Therapy, Myoblast, Body Building, Beautification, Cosmetology

1. Introduction

Aging is hereditary [1]. It is characterized with progressive cell degeneration and tissue attrition, leading to organ failure and death. Myoblast Transfer Therapy (MTT) is a genetic cell therapy which replenishes dead myofibers with live ones, and repairs degenerative myofibers with normal complimentary genes [2]. MTT prevents organ failure, intervening with disease onset and/or progression, thus prolonging the life expectancy of the human subjects [3]. By maintaining normal function of various bodily organs in which myofibers are a constituent, MTT sustains the human subject in good health with a good quality of life [4]. It is an area with great promise that can be traced to one of the earliest INDs of cell therapy and gene therapy, numbered BB-IND 5108, approved on May 13, 1993 by the Food and Drug Administration (FDA) in facilitating the availability of safe and effective treatments [5].

2. Pathogenesis and Signs of Aging

The earliest signs of aging are loss of energy and strength due to genetically programed degeneration of ATP-producing mitochondria (**Figure 1**) [4]. Mitochondrial degeneration diminishes energy production, body warmth, physical activity, leading to disuse atrophy and degeneration of fast-twitch (Type II A and B) muscle fibers [6] that account for slow movement and incoordination in the elderlies. Tendon origin and/or insertion of atrophic muscles are often unduly overstretched causing excruciating pain to the subject. Weakening of anal and urinary sphincter muscles can even affect excretory function. Women after two to three natural childbirths may experience stressed urinary incontinence (SUI) just like men after surgical removal of hypertrophic prostates. Perhaps the loss of muscle bulk and strength is the most significant deficit of aging. Whereas this loss is socially accepted as a normal trend of physiologic development, it is this deficit which hinders the subject to continue to work, socialize, or to participate in professional sports.

Losses of muscle fibers and physical activity are a vicious cycle affecting not only skeletal muscles but the overlying skin also. Reductions in muscle volume coupled with constant muscle stretching and relaxation are direct causes of fine lines and wrinkles on aging skin which begins to lose elasticity, smoothness, suppleness and firmness. This aging process of the skin is accelerated with long exposure to the ultraviolet sunray (photo-aging) and to dry wind. Stress, lack of sleep, bad habits and diets all add to it. The aged skin develops age spots and actinic keratosis with accumulation of melanocytes, a rough non-homogeneous stratum corneum and a thinner dermis. These serious alterations in skin structure often cause change in membrane permeability to various ions in the sensory nerve endings embedded in the skin surface, causing allergy and itching. Skin fibroblasts usually undergo mitotic divisions to cope with adverse conditions in attempt to maintain homeostasis. However, their lack of substantial regenerative capability as limited by their telomere content forces the oncogenes to be turned on and results in skin cancer.

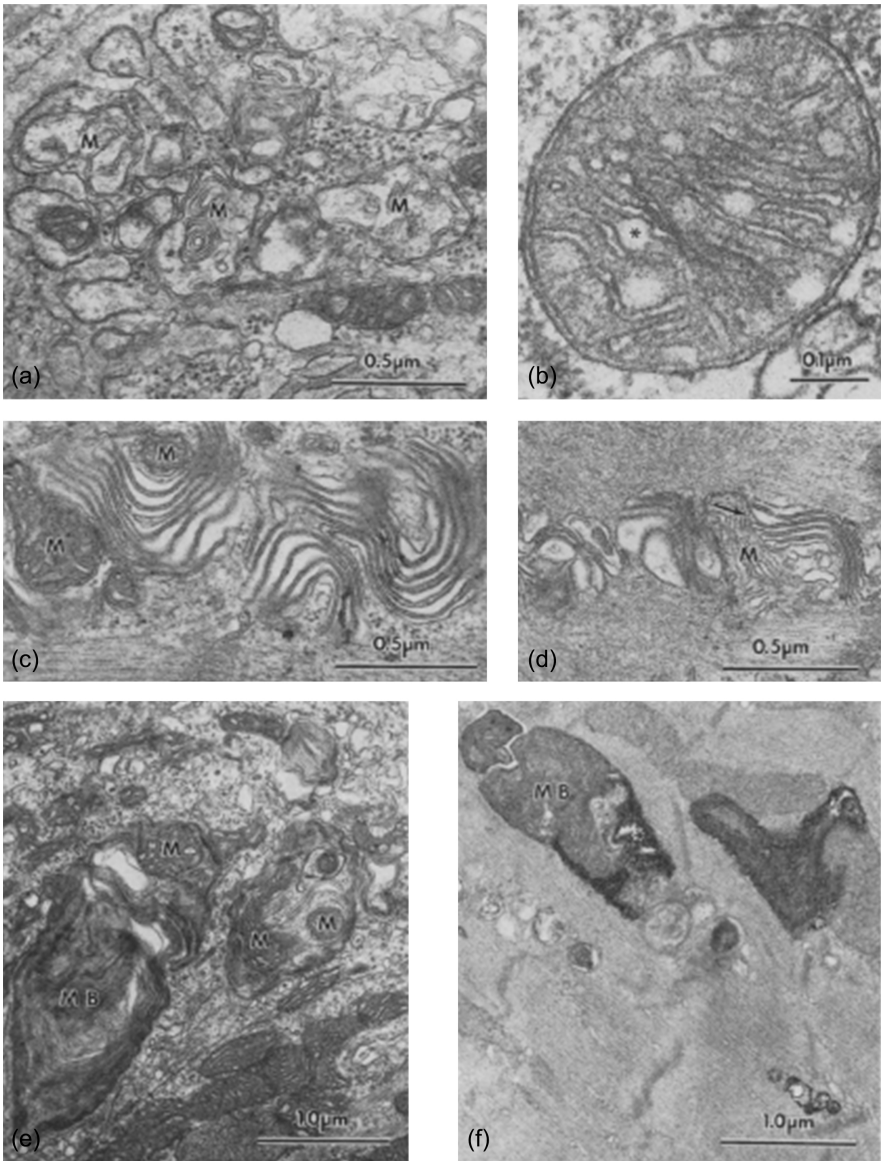

Figure 1. Electron micrograph showing abnormal mitochondria (M) and membranous bodies (MB). (a) Section of degenerative muscle fiber containing membranous structures could still be recognized as swollen and disrupted mitochondria. (b) Mitochondria with numerous areas of low density (*) in the matrix space. (c) Membranous body associated with mitochondria. (d) Mitochondrial outer membrane forming a continuum (T) with membranous body. (e) Large autophagic vacuole or membranous body containing membranous structures and mitochondria. (f) Membranous bodies showing positive acid phosphatase reaction. The asterisk denotes a lead phosphate deposit. (Reproduced with permission from Law *et al.* Experimental Neurology 80: 361, 1983. Academic Press, New York).

Addiction of coffee (caffeine), tea (nicotine), alcohol and/or drugs significantly reduces calcium and magnesium ion absorption leading to osteoporosis, bone fracture and premature loosening and loss of teeth. The latter is often associated with gum atrophy. The greatest storages of these ions in the body are in the sarcoplasmic reticulum of muscle fibers and the blood stream.

The blood circulatory system, especially arterioles, venules, and capillaries become construed or brittle with age and break, leading to cell death as in ischemic heart disease, stroke, and in Type-II diabetic patients with ischemic skin which necessitates skin grafting or even amputation. Central to capillary blockade and breakage is the loss of oxygen and nutrients to the end-organs, resulting in hair loss, erectile dysfunction, pale face, and significant reduction in regenerative activity and capability.

Mitochondrial degeneration in brain cells causes lack of instant recall, even long-term memory. Death of neurons, Schwan cells and astrocytes lead to lacunar infarction of cerebral cortex, brain stem and cerebellum resulting in brain atrophy.

The structural and functional losses all contribute to distortion of the former youthful appearance and joyous psycho, ultimately distressing the subject and in severe situations result in depression, and schizophrenia.

As such, aging is a hereditary, chronically debilitating and ultimately fatal disease. Anti-aging is a medical science different from cosmetic cover-ups. It conveys great social values in contrast to the general perception of its just having economic values. The gross abuse of scientific understanding without demonstrating the safety and efficacy of treatment procedures for lucrative financial gain constitutes medical malpractice.

3. Market Size

The baby-boomers after the Second World War are over 75. They will be the prime users of AARC. The world revolves around the achievers who are usually over 35. There are numerous body builders and beauty users in their twenties and even late teens. Then there are those with birth deformities, trauma, burns, surgical wounds, malnutrition and others. It is estimated that more than 50% of the world population now needs some form of AARC treatments. With exponential growth of cell therapy technologies in regenerative medicine, human beings will live past 100 years before this century ends. These elder and wiser people will demand better appearance and better quality of life. In time, almost every living human being will need one or more of such enhancement with age.

4. Beauty and Its Parameters

Beauty is a physical attribute that often enhances one's self-confidence, career and quality of life. Whereas there is a common perception about what constitutes beauty, beauty is truly in the eye of the beholder. Advances in artificial intelligence and 3D-typing allow computerized animation for the subject's body part to be modified to the liking of the subject. The ability to control and modulate the various physical parameters of appearance using the genetic cell therapy of MTT ensures customized satisfaction.

The physical parameters of appearance are **size, shape, tone, color, luster, texture, consistency, and density.** These inborn characteristics may not satisfy the subject's fancy. These parameters deteriorate in every external organ ac-

cording to the genetically programmed degeneration of aging. Throughout the ages, there are numerous anti-aging ways using non-living ingredients. Current technologies favor human cell therapies using live cells, cytokines and co-factors to enhance the parameters of appearance. These biologics, as they are called, are designed to provide improvement of appearance and function.

5. Cell Therapy Cosmetics

The cell is the basic unit of all life. They are the building blocks of the bodily organs. Good health is the well-being of all bodily cells. Cells are what life is made of. In aging or degenerative conditions, cells degenerate and die, rendering the organ (e.g. hair, skin, muscles, breasts, sex organs) which they inhabit dysfunctional. An effective treatment must not only repair degenerating cells, but also replace dead cells with live ones as well. This can best be achieved by the transplantation of genetically normal cells, or cell therapy [6].

Cell culture in the laboratory is the only method known to man for the replication of new live cells. With proper *in vitro* techniques and precautions, normal cells can be cultured in compliance to standards to repair degenerates and wounds, and to replenish the degenerative organ with new live cells [7].

Cell transplantation allows propagation of "new life" in degenerative tissues. The dysfunctional organ, once invaded with scar and necrotic tissue, is now repopulated with live cells that augment its appearance (**Figure 2**) and function (**Figure 3**).

6. Myoblast Anti-Aging Therapy

Human beings are products of evolution. In the last 160 million years of mammalian evolution, skeletal muscles have developed to occupy 55% of the volume of the human body. With every muscle fiber containing 200 to 500 nuclei, and approximately 100 mitochondria adjacent to every muscle nucleus, MTT is the best regenerative technology to rejuvenate the body by providing DNAs and ATP. No other tissue in the human body exhibit a higher density of nuclei and mitochondria, the organelles that harbor DNAs and ATP for production of strength and energy (**Figure 4**). Thus, the FDA approved Phase II and Phase III 50-billion-MTT protocol that was demonstrated to be safe and efficacious in human clinical trials [8] literally implanted 50 billion muscle nuclei and 5 trillion mitochondria into 80 large muscle groups of single Duchenne muscular dystrophy (DMD) boys and limb-girdle muscular dystrophy (LGMD) adults. Many of these subjects demonstrated significant improvement in quality of life and life prolongation [4]. They represented a small group of seriously genetically defective individuals saved by MTT. The same technology can be applied to anti-aging in improving muscle structure and function. Advancements in tissue-resident adult stem/progenitor cell research have revealed that enhanced telomere attrition, oxidative stress, ultraviolet radiation exposure and oncogenic events leading to severe DNA damages and genomic instability may occur in these immature and regenerative cells during chronological aging [9].

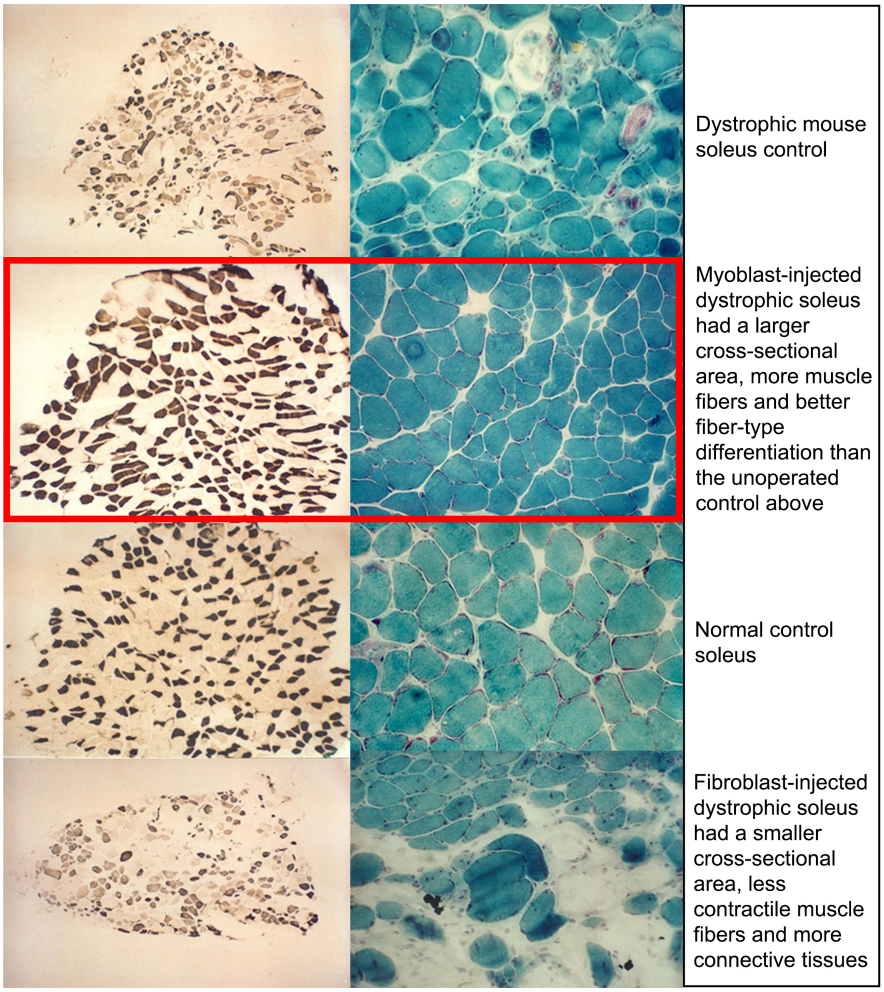

Figure 2. MTT augments size of treated organ by increasing muscle fiber number and tonicity, adding speed with increase in fast-twitch muscle fibers (Reproduced with permission from Law *et al.* Muscle Nerve 1988; 11: 525-533).

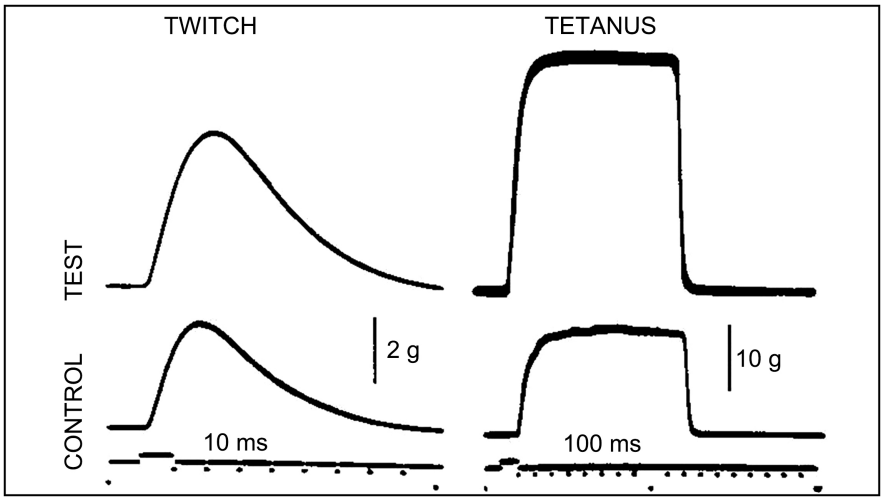

Figure 3. Myoblast-injected dystrophic soleus showed significant increases in twitch and tetanic tensions than the contralateral un-injected dystrophic soleus (Reproduced with permission from Law 1994. RG Landes, Austin).

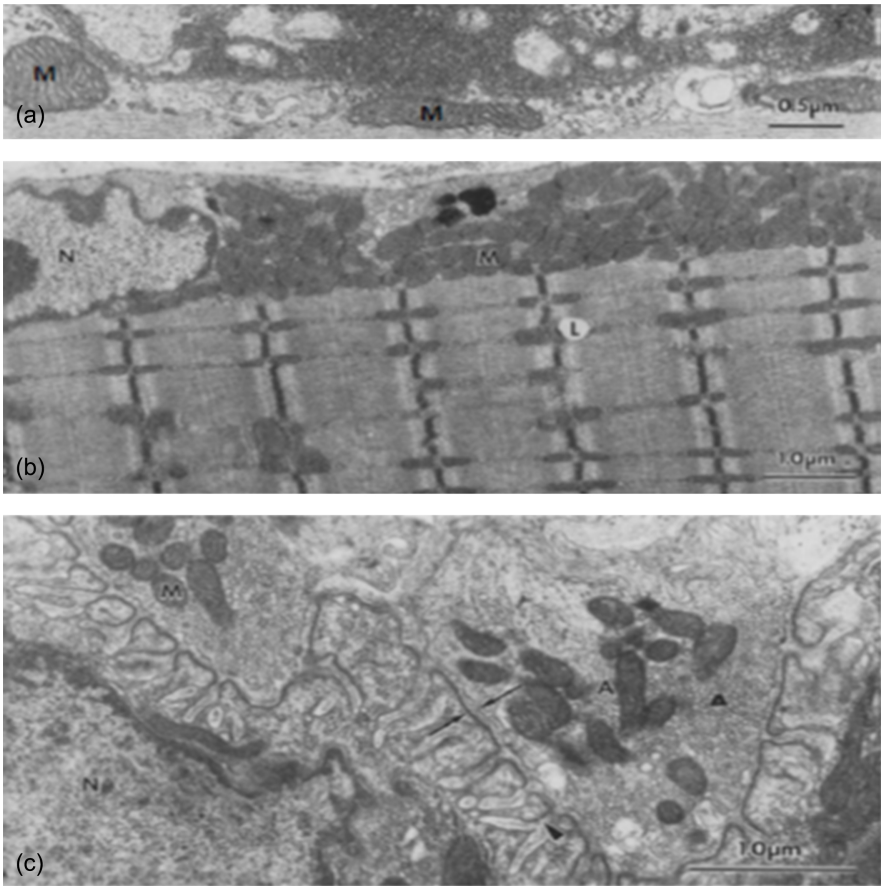

Figure 4. Electron micrograph showing (a) Normal appearing mitochondria - M with cisternae regularly spaced; (b) For every nucleus, there were about 100 mitochondria around; (c) Normal presynaptic terminal or axon-A containing numerous mitochondria-M (Reproduced with permission from Law *et al.* Experimental Neurology; 80: 361, 1983 Academic Press, New York).

The feelings of being weak and cold during aging indicate degradation of the energy production network of mitochondria (**Figure 1**) [10] densely populating the neuromuscular systems that are doing the real heavy lifting (**Figure 4**). Striated muscles, cardiac muscles and smooth muscles that occupy approximately 55% by volume of the human body are constantly at work to maintain body temperature and normal metabolism. Degradation of the power network of adeno-triphosphate (ATP) production leads directly to cell apoptosis, organ malfunction and eventually human death [4].

With the 50-B MTT into 80 large muscle groups of the body, there was a mean 70% increase in normal myofiber number attributed to donor myoblast fusion after transplantation [8]. Considering that each myofiber contains 200 to 500 muscle nuclei, literally trillions of mitochondria from young men are implanted with each 50-billion MTT to regenerate the energy network of life. These are not isolated and manipulated mitochondria that have difficulty in integrating into the human body through mitochondrial transfusion [11]. The MTT protocol was the one that has kept some DMD patients living in their 30's and 40's in-

stead of expiring at age 20 [3] [4] [12] [13]. It may have similar effect on the elderlies and Type-II diabetics [14].

The proof-of-concept presented above indicated that intramuscular injection of myoblasts can augment the size, shape, consistency, tone, and strength of muscle groups, improving the lines, contours, and vitality from the sculpture for a youthful appearance. Table 1 illustrated the gradual increase in muscle strength as contractile filaments like actin, myosin, troponin and tropomyosin were deposited and organized after the myotubes were vascularized, innervated and matured to be muscle fibers. An average 123% increase in maximum contractile force at 18 months after MTT was obtained taking the means from four major muscle groups of the body. The results were based on studies of 50-B MTT on DMD muscles. Given the treatment, one would expect the elderlies, the athletes, the soldiers and the astronauts will out-perform the DMD boys in these increases in muscle contractility.

7. Facial Rejuvenation

The face identifies an individual. It is the first impression to a stranger. For love of beauty, an inborn beautiful or handsome face often bestows and conveys its owner special opportunities and benefits, especially in life-long events such as career, marriage and friendship that are linked directly to one's happiness. Thus, it is not surprising that not only the rich and powerful but innumerous well-to-do people have become addicted to facial rejuvenation. With the baby-boomers after the Second World War all approaching age 75, facial rejuvenation becomes a norm for the 962 million people over age 60. This market size, however, is nothing compared to that of users with age spanning from 25 to 65.

8. Biologic Skin Cover

Cell replacement and targeting of the molecular systems found in skin hold great promise for controlling or even curing skin aging [15]. Myoblasts and foreskin fibroblasts are differentiated cells committed to their lineages. They are capable of extensive cell division in culture. The two cell types can be used to develop

Table 1. Mean percentage increases in the maximum contractile force of the plantar flexor muscles of DMD boys at 3, 6, 9, 12 and 15 months after the administration of the 25-billion MTT protocol (800 million myoblasts injected) or the 50-billion MTT protocol (1200 myoblasts injected) (Reproduced from Law *et al.* Gene Therapy and Molecular Biology, 1: 345-363, 1998).

	Months after MTT				
	3	6	9	12	15
25 Billion MTT (800×10^6 myoblasts)	9%	19%	31%	45%	61%
25 Billion MTT (1200×10^6 myoblasts)	11%	23%	37%	53%	71%

into body cover, either singly or in combination. Myoblasts, because of their small size, spindle shape, and resilience, can grow within wrinkles and on collagen and laminin of skin surfaces without blood supply, thus enhancing the color, luster and texture of the skin "plated" with them (Figure 5). They can have anti-inflammatory and antiapoptotic properties by paracrine-secreting growth factors and cytokines on the site of administration [16].

The common skin fibroblasts are polygonal, 10 to 15 times the size of the myoblasts and produce a rougher body cover. Cancers are commonly associated with skin fibroblasts but not with myoblasts and foreskin fibroblasts. Thus, a new layer of biological skin consisting of pure myoblasts and/or foreskin fibroblasts can eliminate skin defects and blemishes. Safe and efficacious for the hands with serious wrinkles, the technology will be applicable for wrinkle and blemish reduction for the face and other skin parts.

A patented method for refurbishing skin of an individual comprises 1) removing dead cells from the surface of the skin with a smooth abrasive or by one or more chemicals such as lactic acid to generate a prepared surface and 2) applying myoblasts in a myoblast cell-nutritive solution to the prepared skin surface. The myoblast cell suspension useful for skin enhancement of an individual, comprising autologous or allogeneic human myoblasts, serum from the individual, an angiogenesis factor and large 6 chondroitin sulfate for controlled, rapid cell fusion of the myoblasts. The invention may also use foreskin fibroblast cell suspension in a similar process, either singly or in combination with myoblasts.

Myoblasts from a serum-like suspension (MYOLA) is applied as thick layer(s) with a sterile brush to the prepared skin surface such that the myoblasts survive, develop, and become integrated into the collagen and laminin of the human skin, thereby filling in cracks, crevices, wrinkles, blemishes, holes and laser scars of the skin. The myoblasts smooth imperfections in the skin and provide further qualities such as resilience, coloration and strength. Warm moist oxygen-containing air, or more preferably pure oxygen is blown onto the treated

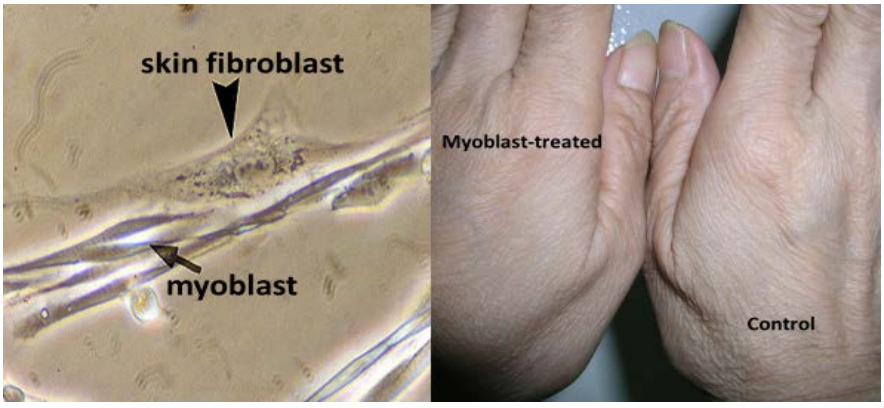

Figure 5. Supra-cutaneous application of adherent myoblasts onto the left hand resulted in wrinkle reduction two weeks after (Reproduced with permission from Law and Law. Recent Patents on Regenerative Medicine 1: 88-117, 2011 [8] Bentham Science, Oak Park).

area for at least 1 hour. Cell graft is left undisturbed for at least 12 or more hours. Thus, layers of biologic skin consisting of pure myoblasts and myotubes can eliminate skin defects and blemishes left behind after laser removal. The whole procedure may be repeated in intervals of 6 to 12 months to obtain smoother and younger looking skin.

Similarly, foreskin fibroblasts may be used singly or in combination with myoblasts and/or myotubes to produce similar results. Foreskin fibroblasts advantageously can provide smooth texture and tensile strength to the new skin. A mixture of myoblasts and myotubes is particularly useful for filling in large skin areas such as large wrinkles. The presence of myotubes helps mechanically bridge large gaps, and the ratio and average size of the myotubes in such mixtures may be prepared and adjusted as needed.

Myoblasts can be autologous obtained from the treated individual, or from other human beings. If not autologous, the immunosuppressant cyclosporine is administered 2 days in advance. Although other immune suppressants may be used, cyclosporine for 2 weeks at two oral doses totaling 5 mg/Kg body weight per day is preferred because it facilitates donor cell survival.

Myoblasts can be also injected subcutaneously as a cellular filler to reduce wrinkles.

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Law PK. Biologic skin repair and enhancement. WO2004017972A1; WO2004017972A8; AU2003263906A1; AU2003263906B2; CA2496434A1; CN100482228C; CN1700915A; CN03819963.7; CN E038199637XS; EP1587515A1; EP1587515A4; SI110581; US2006057119A1.

9. MYOLA (Biologic Skin Cream)

Biologic creams are formulated to promote cell survival, growth and development to enhance the color, luster, density and texture of the skin. Fair skin is desirable, but its exposure to radiation of strong sunlight often leads to skin cancer. Myola, a unique essence formulated with myoblast extracts, is specially designed to rejuvenate the fair skin. Among other ingredients, it comprises

1) tumor necrosis factor alpha (TNF-α), an anti-cancer and a whitening factor;
2) telomeres [17], a mitotic agent to enhance cell division;
3) mitochondria, the ATP manufacturer to provide energy for regeneration;
4) muscle nuclei, the DNA supplier of regenerative proteins for cell repair.

In additional to being used as a carrier solution in the skin refurbishing mentioned in the last section, MYOLA will find application in daily skin maintenance.

Endogenic and exogenous exosomes are being developed as treatment options to repair, regenerate, and rejuvenate skin [18]. The paracrine factors of stem cell-derived exosomes have significant potential as a cell-free therapy for scar treatment [19].

10. Soft Tissue Augmentation

Intramuscular injection of myoblasts can augment the size, shape, consistency, tone and strength of muscle groups, improving the lines, contours and vitality from the sculpture for a youthful appearance [3]. The myoblast technology can be used for cosmetic enhancement such as bodybuilding and in tissue implants for breast/buttock/facial augmentation or reconstruction (**Figure 6**, **Figure 7**) [3].

The myoblasts can be injected intramuscularly to grow muscles, or subcutaneously as a filler. Unlike the noncellular collagen which will be absorbed in three to six months after injection, injected myoblasts are cells that will survive and last for tens of years within the host. Myoblasts are endogenous to the human body and have been proven safe in clinical trials involving over 300 muscular dystrophy patients and 300 heart patients worldwide [3]. Myoblasts will not cause cancer like silicone. Their constructs will not burst and be absorbed like saline or collagen implants. They develop to become myotubes and muscle fibers.

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Law PK. Myoblast therapy for cosmetic treatment. WO9618303A1 US7341719B1; AU4597696A; CA2183167A1; CN03101588.3; CN1127343C; CN1146712A; CN1477190A; EP0743820A1; EP0743820A4; EP1407788A2; SG74036A1; SG99279A1; SG99846A1; US7341719 B1.

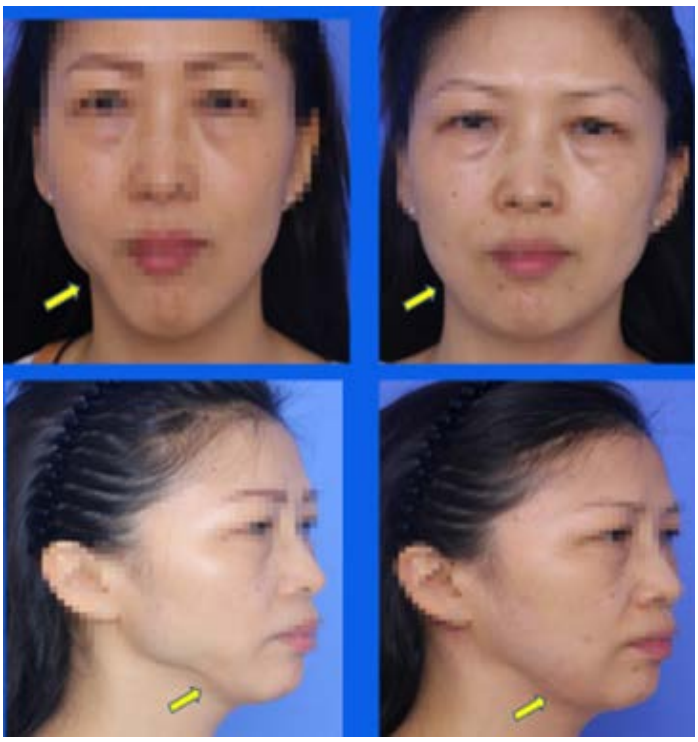

Figure 6. Facial restoration with 10 billion myoblast-implantation before versus after 3 months.

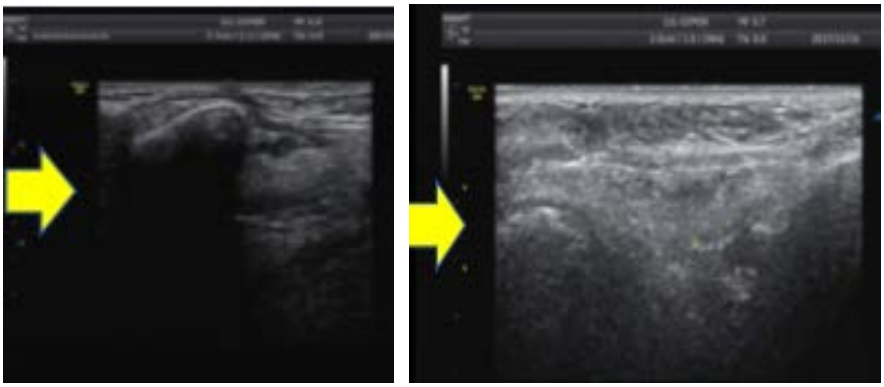

Figure 7. Ultrasound examination demonstrated that subcutaneous soft tissue thickness increased from 0.30 cm before implantation (left) to 1.35 cm 3 months after (right).

11. Aging Diabetics

Aging diabetics will be the ideal subjects to be tested on the Genetic Cell Therapy for Anti-Aging Regenerative Cosmetology of MTT because they suffered the combined pathological consequences of both aging and Type II diabetes. In the world's first human gene therapy and somatic cell therapy [2], it was concluded, "Through natural cell fusion, which is inherent in myogenesis and muscle regeneration, donor myoblasts insert full complements of normal genes into dystrophic muscle fibers. Therefore, it does not matter which gene is abnormal or which protein is missing. MTT has potential application to all hereditary muscle diseases. It repairs degenerating cells and replenishes lost cells. This first case suggests that MTT offers a safe and effective means for alleviating muscle biochemical deficit(s) in muscles of DMD." This conclusion was extrapolated to a Type II diabetes clinical study testing for feasibility and safety [20], and to diabetic mouse studies for pathogenesis, mechanisms and proof-of-concept [21] [22].

The mouse studies indicated that xenotransplantation of normal human myoblasts into limb skeletal muscles of KK diabetic mice up-regulated multi-genes involved in impaired insulin signaling pathway and mitochondria biogenesis and function. This was accompanied with improved glucose tolerance and reduced hyperglycemia in the KK mice. The results indicated that MTT regulated the expression levels of genes that play important roles in the pathogenesis of skeletal muscle insulin resistance.

It is anticipated that, in addition to providing a genetic cell therapy to relieve the various symptoms of Type-II diabetes, the 50-billion MTT will instill biochemical, structural and functional improvements to the aged or aging subjects. It is long overdue to conduct clinical trial on this large population of aging diabetics using MTT, considering the devasting sufferings of the ill and the huge social economic burden borne by the national healthcare systems.

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Law PK. Disease prevention and alleviation by human myoblast transplan-

tation. US13/968,982; US10449219B2; US2015050300A1; US2018000867A1; AU2013231029A1; CN201310455357.4; CN103550784A; CN107648267A; CN108042571A; EP2837683A1; EP2837683B1; ES2615553T3; HK1248114A1; JP2015051969A

12. Anti-Aging Angiomyogenesis

Distribution of oxygen and nutrients to the peripheral organs is significantly reduced for people aged over 45. In developing treatment for human myocardial infarction, we have grown five times more blood capillaries and muscle fibers simultaneously using human myoblasts transduced with human VEGF (vascular endothelial growth factor) gene [20] [23] [24]. In addition to their application to treating heart diseases [25]-[30], potentially these cells can be used to treat male/female impotency, baldness and to produce redder lips and pinker face because of the higher density of capillaries within layers of myogenic cells after myoblast treatment. The latter serves as a fertile ground to seed new hair follicle cells on the bald head or other body parts to give the desirable hair color, density and consistency. The effect of increased vascular reactivity may be useful in pathological situations in which an intense angiogenesis is desirable, such as tissue ischemia [31]. Likewise, the angiomyogenic myoblasts can be chemically transformed using bone morphogenic proteins (BMP) to become osteoblasts [32] for treating osteoporosis, bone fractures and loose tooth pulps (**Figure 8**).

13. Bioactive Implants and Fractal Stimulation

1) Coating of implantable materials with myogenic cells

Allogeneic and autologous myoblasts are particularly useful for coating biological body parts, for example a pig heart valve, human damaged tissue, and non-biologics such as stents, plastic valves, plastic heart valves, vessels either

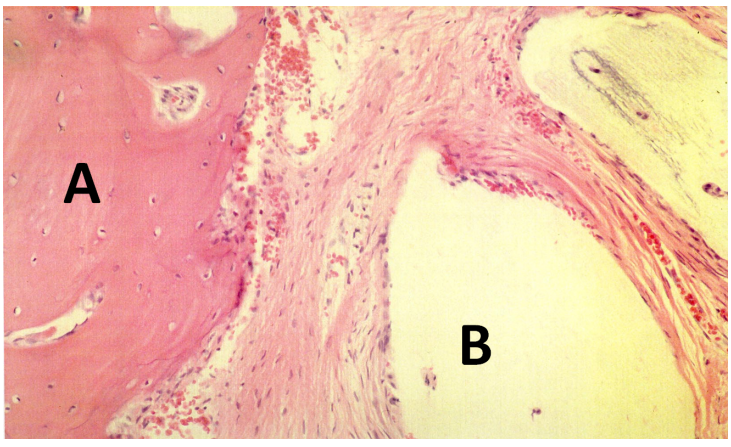

Figure 8. Transverse section of a part of a rabbit skull showing: (A) Bony reconstruct filling a small hole drilled on the rabbit skull 6 months ago that was implanted with a mixture of human myoblasts, bone morphogenetic protein (BMP) and hydroxyapatite (HA); (B) A similar hole implanted with BMP and HA but no myoblasts showed no bony reconstruct and was left unfilled. H&E stain. Microscopic magnification, 400×.

biologic or synthetic, and any other implantable materials. A biological tissue in this context may be for example, a sphincter muscle, a skin, a tongue, an eyelid, an eardrum, stomach tissue, bladder tissue, vascular tissue, artery section, vein section, aorta section, heart tissue. A skilled artisan can optimize the amount and conditions for coating.

For example, a pig heart valve may be coated with myoblasts obtained from a muscle biopsy derived from the patient. Desirably, the pig heart valve, or other implant is first coated with a scaffold material such as fibronectin, laminin and/or collagen prior to coating with myoblasts. Desirably, the coating is thick enough to block sites of the uncoated surface from reacting with one or more components of the immune surveillance system. Crosslinking adsorbed material may be carried out by any of known conventionally means to both ensure long term attachment and a suitable thickness.

2) Implantation of Myogenic Cells, Particularly Autologous Cells for Enhanced Muscle Function

Many patients that need electrode stimulation enhancement of muscle function have weakened muscles. These muscles acquire improved function by transplanting myogenic cells before, during or after electrode implantation. Myogenic cells, such as autologous myogenic cells prepared from the patient beforehand, can be reintroduced at high levels, such as 10^6 to 10^7 cells, 10^7 to 10^8 cells, 10^8 to 10^9 cells, or even higher. The cells, once introduced, fuse with preexisting muscle cells, thus contributing their DNA and fortifying the bulk of the native muscle.

3) Synergy results from including myogenic cells with an electrode. The myogenic cells, when already attached to an electrode, shorten the time required for the body to accommodate the electrode. A smaller excitation current is required because of the accommodation of electrode with muscle cells *in vitro*, particularly when the muscle cell morphology is altered prior to implantation with the use of entraining currents *in vitro*.

For example, cells that coat the electrode and other cells in the vicinity, in an embodiment, are electrically stimulated by the electrode during growth in culture. The stimulation may occur over a period of at least 1 hour, 3 hours, 6 hours, or 12 hours but preferably occurs for at least one day, two days or three days. This stimulation may facilitate the induction of genes involved with formation of the motor end plate and/or other features that desirably interact with the electrode. By increasing the amount of these features, a more sensitive electrode response may be elicited after implantation.

In another desirable embodiment, nerve cell progenitors likewise are incubated with the electrode *in vitro* before implantation. In a desirable embodiment, nerve cells precursors and myogenic cells are incubated with the electrode *in vitro* and experience electrical activation of that electrode *in vitro*, which induces the cells to form structures that respond to the electrical activation. The amount of electrical current and voltage used as well as periodicity and wave form, preferably similar to that used on the electrode after implantation. Prefer-

ably the stimulation is biphasic, to alleviate effects of electrolysis, and the instantaneous current is less than 10 ma, more preferably less than 3 ma, and in some situations where repeated stimulations (more than once per 5 minutes) occur over an extended time, less than 0.5 ma or even less than 100 microamperes.

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Law PK and MOTSENBOCKER MA. Bioactive implants. WO2004030706A2; WO2004030706A3 AU2003272805A1; AU2003272805A8

Therapeutic stimulation methodologies based on inherently fractal nature of physiologic dynamics involve the use of electrical currents, electromagnetic fields, temperature change, ultrasound, light, and so forth. These stimulation therapies can be categorized into three main modalities: electrical stimulation modalities, thermal modalities, and non-thermal modalities. Electrical stimulation modalities include therapeutic techniques where electrical current is directly applied to the body of treated subject. Direct application of electrical current to the brain also falls under this category. Thermal modalities consist of stimulations that induce temperature change on the body for therapeutic effects without the direct transfer of electrical current. Non-thermal modalities functions through energy transfer without directly applying electrical current and without the effects of temperature change. A fourth miscellaneous category for stimulation techniques consists of the stimulation effects of music along with physical stimulation as in massage therapy. Common to most of these therapeutic strategies is that the stimulation is delivered at certain fixed periods or frequencies. We introduce some rudiments of fractal dynamics, and the notions of self-similarity, scale-invariance, and long-range correlation or memory in the dynamics of a system [33]. We present evidence that fractal dynamics is commonly observed in healthy physiological systems while unhealthy systems are shown to veer away from fractal dynamics towards periodic or random motion. This difference in dynamics can be observed in many biological signals such as in neural activity, heart rate variations, and breathing patterns. We propose that an optimal stimulation technique should thus be one that encourages an unhealthy, non-fractal pathological system towards a healthy, fractal dynamic. Given the ubiquity of fractality in healthy biological dynamics, we argue that a fractal pattern of stimulation is a more optimal approach to functional restoration than the widely used conventional periodic stimulation, which may further consolidate the existing pathological dynamics [33].

14. Stressed Urinary Incontinence (SUI) and Fecal Incontinence (FI)

These are embarrassing dysfunctions that inhibit socializing of innumerous numbers of women aged 45 upward. Birth of children has left these ladies, some holding important offices, with weakened internal and external sphincter mus-

cles. Publication over 1100 cases has demonstrated that MTT is safe and efficacious for SUI and FI. In conjunction with treating SUI, more myoblasts can be implanted into the vaginal wall to enhance tightness and contractile force during sexual intercourse. Current surgical and cauterizing techniques often left scars that created discomfort for both partners. Myoblast implantation over the pubic bone provides not only a sexier appearance, but also a "shock absorber" on impact, thus reducing the piercing discomfort caused by the skinny yet attractive ladies. And for that special partner and moment, a hymen can be reconstructed from layers of myotubes cultured and patched on with fibronectin or biologic glue. Similar restructuring of the larynx can produce a sexier voice of higher pitch, a tympanum for the traumatized eardrum, a cellular patch for esophageal and/or stomach ulcers.

15. Tendon Repair in Sport Medicine

Myoblasts constitute a better biomaterial than tenocytes in synthesizing stronger and smoother tendons for transplantation repair. These tendons exhibited greater stiffness, tensile strength, Young's modulus, and could handle higher maximum load [34]. One can envision MTT developing into sport and military medicine in muscle, tendon, bone, cartilage and joint repairs.

16. Tai Chi and Chi Gong

No matter how good a muscle is endowed, it needs exercise to be in shape. In younger days, active sports provide competitive spirit and release of endorphins and hormones essential for maintenance of youth. In addition, muscle stretching such as in yoga induces new formation of sarcomeres, thus increasing the maximum contractile force. As one ages, active sports may over-stretch tendons, fracture bones and rupture capillaries.

Tai Chi and Chi Gong are milder forms of exercise recommended to people aged 45 and over. The movements are slow and are designed to provide isotonic muscle contractions rather than purely isometric. Historically, these are two styles of Kung Fu developed by Chinese martial Taoists to maintain balance of body and mind. Tai Chi strengthens the postural muscles of the waist and the legs, thus helping to prevent the elderlies from falling. Chi Gong increases the oxygen content of the body by training and strengthening the respiratory muscles such as the rectus abdominis, the oblique, the paraspinal muscles and the diaphragm in order to shift the ordinary thoracic breathing into the adapted abdominal mode.

By willfully depressing the diaphragm and filling the lungs, one can increase oxygen intake. The mild and sustained movements of Chi Gong also enhance oxygen distribution via the blood circulatory system to various extremities of the body including the brain. The persistent inhaling and exhaling exercise train the body to develop low oxygen tolerance and strong nasotracheal muscles.

Chi Gong has immense value for the wheelchair bound and bed-laden elder-

lies and patients. In addition to preventing disuse atrophy and oxygen deprivation, Chi Gong provides the trained ability to cough up mucus from the trachea which proves to be invaluable in saving lives at times of pneumonia, COVID-19, and respiratory failure in DMD. Half an hour of Chi Gong a day chases the death spirit away.

Many years of feeding often leave the alimentary canal stretched with fauna of bacteria and fungi. The latter feed on carbohydrate and fibers, generating large amounts of carbon dioxide and hydrogen sulfide that are toxic to the body. Elderlies lacking exercise usually have gas entrapments in the intestine, causing distension and constipation. The intraabdominal pressure often leads to high blood pressure and heart ischemia. Tai Chi and Chi Gong are perhaps the best forms of exercise for the elderlies to relieve the yearnings for "Porsche" and "Ferrari", though one is sitting in a Rolls Royce or Mercedes Benz.

17. Familial Myoblast Cell Banks

A myoblast cell line of 100 billion or more can be established from 2 grams of muscle biopsy from the healthiest male child of each family, to be used in the prevention, treatment or beautification of every family member.

18. Conclusion

The genetic cell therapy of MTT regenerates aging muscles and skin to provide added strength and youthful appearance. Implanted myoblasts, preferably allogeneic, survive, develop and function in the host with 2-weeks' oral doses of an immunosuppressant. The myoblasts develop into muscle fibers, adding 70% new muscle cells to augment the size, shape and function of the injected muscle or organ. Intramuscular injection of 50 billion myoblasts into 80 major muscle groups under general anesthesia provides huge amounts of nuclei and mitochondria that are necessary to operate the regenerative processes. The inherent natural cell fusion of myoblasts incorporates nuclei transfer, foreign gene integration, regulation and expression all in one encounter to ensure natural spatial and temporal transcription of the normal genome to produce whatever missing proteins that are necessary to be young again. Future directions include the concept of "precision cosmetic medicine" utilizing gene editing and cellular therapies to tailor rejuvenation techniques based on individual's genetic make-up and therefore needs [35]. Anti-aging regenerative medicine is as exciting as ever!

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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