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Cardiac pre-differentiation of human mesenchymal stem cells by electrostimulation

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1. ABSTRACT

Myocardial repair using stem-cell therapy has become a promising therapeutic tool. However, many questions concerning a precise functional integration of injected cells remain unanswered. The use of cardiac pre-committed cells may improve integration, as these cells may complete their differentiation in the myocardium reducing fibrosis and restoring muscle function. We have previously demonstrated that electrostimulation (ES) induces cardiomyocyte pre-commitment of fibroblasts *in vitro* and is an effective alternative to cytokine-induced differentiation. In this study, we evaluated the effects of long term electrostimulation on human mesenchymal stem cells (hMSCs). ES induced both morphological and biochemical changes in hMSCs resulting in a shift toward a striated muscle cell phenotype expressing cardiac specific markers. This partially differentiated phenotype might allow a gradual, ongoing differentiation within the cardiac environment, providing time for both myocardial regeneration and electro-mechanical integration, and convey potential advantages in clinical applications.

2. INTRODUCTION

Left ventricular remodeling is associated with myocyte apoptosis and replacement of myocytes by fibrous tissue in the ventricular wall. This is considered to be at the root of heart dysfunction after an acute myocardial infarction. At the cellular level, sudden suppression of the oxygen supply triggers intricate molecular cascades which modulate a series of critical biological events (1). Immediately after an infarction, the process of wound-healing begins with active migration of inflammatory cells, recruitment of cardiac fibroblasts and eventual remodeling of the extracellular matrix aiming to stabilize the region and restore ventricular wall function. However, function is seldom restored, and cardiomyocyte loss and replacement by fibrous elements results in a natural process evolving from progressive left ventricular remodeling to congestive heart failure, a major public health problem (2).

Stem-cell based therapeutic strategies aiming to restore myocardial cellularity and regenerate the contractile tissue have raised considerable interest due to encouraging

preliminary results (3, 4). However, many issues concerning the ultimate fate and precise functional integration of injected stem cells remain unknown. A functional engraftment is required to augment synchronized contractility and avoid potentially life-threatening alterations in the electrical conduction of the heart (5, 6). Current evidence suggests that skeletal myoblasts or bone marrow-derived adult stem cells fail to electromechanically integrate into the recipient heart with direct consequences on their survival and terminal differentiation (7). Thus, identification of cell types which can achieve efficient electromechanical integration is mandatory. Preliminary data suggest that cells with a true cardiomyogenic phenotype, such as cardiac stem cells and cardiac-pre-committed embryonic stem cells may satisfy these criteria and thus, potentially ensuring regeneration of dead myocardium (8). In fact, a partial differentiation or a pre-committement of stem cell towards cardiomyogenic phenotype might be preferable to a fully differentiated cardiomyocyte in this context, as the cardiac environment could provide the necessary biochemical and mechanical signals for integrated differentiation. Following completion of their differentiation within the myocardium, the pre-committed cells could effectively and selectively reduce fibrosis and restore muscle function.

It is possible to induce differentiation of several cell types, including fibroblasts, bone marrow-derived stem cells, human mesenchymal stem cells and embryonic stem cells, into committed cells expressing markers characteristic of a cardiac phenotype (9-11). Unfortunately, these transdifferentiation protocols either rely on the use of powerful and potentially dangerous drugs (11) or require periods of co-culture with human or rodent adult ventricular myocardiocytes. The former clearly prohibits translation to the clinic and the latter raises issues concerning cell survival and the effects of a prolonged *ex vivo* culture on cell immunogenicity and transformation (12). In order to circumvent these key limitations, we recently developed a cytokine-free system using cell electrostimulation (ES) as an alternative mean of inducing pre-commitment or conversion of fibroblasts to a myocardial phenotype. We demonstrated induction of cardiomyocyte pre-commitment in two fibroblast cell lines with different evolutive potential (13).

Mesenchymal stem cells (MSC) are promising tools for regenerative medicine and have been applied in numerous experimental and preclinical studies (14-16). For cardiac repair, especially following myocardial ischemia, MSCs provide an attractive potential therapeutic approach (17). Latissimus dorsi muscle flaps, preconditioned by long term pacing for subsequent use in cardioplasty, undergo cardiac-like differentiation (18). Differentiation to a cardiomyocytic lineage must include gap junction formation, which should, in turn, improve cell engraftment (19) and reduce the risk of arrythmogenic events (20). In our previous report, we demonstrated the induction of cardiac-specific gap junction proteins, cardiomyogenic nuclear transcription factors and cardiomyogenic cytoplasmic filaments following *in vitro* electrostimulation of fibroblasts (13). Given these findings, we decided to investigate the effect of ES on the viability and differentiation of MSCs.

3. MATERIALS AND METHODS

3.1. Cell culture and es protocol

Human Mesenchymal Stem Cells (hMSCs) were purchased from Lonza (Switzerland) and cultured in Alpha-MEM (Lonza, Switzerland) containing 10% fetal bovine serum, 15% NaHCO3, 4 mM L-Glutammine and 100 U/ml Penicillin and 100 ug/ml Streptomycin. Low-passage cells were cultured until reaching 80% confluence, in order to avoid spontaneous differentiation, seeded in four-well plates (NUNC Apogent Rochester, NY) at a density of 5000/cm^2, and left overnight.

Cells were stimulated with a four-channel C-Pace chronic stimulation unit (IonOptics Co., MA). Based on the previously reported voltage dose-cellular toxicity response curve (13) and on the parameters used to stimulate latissimus dorsi muscle for ventricular cardiomyoplasty (21), a voltage level of 10 V was administered in 5 ms pulses and a burst rate of 0.5 Hz. Cells were recovered after 1, 2 and 3 weeks of stimulation. Equal numbers of cells were seeded in separate plates, not treated with ES, and recovered at same time points for use as controls. Immediately after ES, the cells were processed for evaluation of cell viability and phenotypic characterization.

3.2. Cell viability

Assessment of cell viability after ES was performed by Trypan Blue exclusion according to manufacturer's instructions (Whittaker, Cambrex, Belgium). A LIVE/DEAD Cell-Mediated Cytotoxicity Kit, based on a double fluorescent staining with ethidium bromide and Ca fluorescein (Molecular Probes, Invitrogen, CA), was also used to assess cell death after ES. Percentage of dead and non-viable cells was calculated by counting at least 200 cells in 5 randomly chosen microscopic fields at 10X magnification.

3.3. Immunocytochemical analysis

Expression of cardiac troponin I (cTnI), sarcomeric actin, desmin and connexin 43 (Cx43) was evaluated by immunocytochemistry and immunofluorescence as previously described (13, 22, 23). For immunofluorescence, mouse monoclonal anti-cTnI (at a dilution of 1:1000) and anti-sarcomeric actin (1:1000), Cx43 (1:500) (Biomeda, CA) primary antibodies were used. The cells were incubated with Alexa Fluor 488-conjugated secondary anti-mouse IgG and Alexa Fluor 546-conjugated secondary anti-rabbit IgG (Invitrogen, CA) for 30 minutes at 37 C. All cell preparations were counterstained with TO-PRO3 (Invitrogen, CA) and cytoplasmic actin was counterstained with phalloidin red (Invitrogen, CA), when necessary. In order to clarify molecular mechanisms underlying protein expression after stimulation, monesin (BD bioscience, CA), was used in some experiments according to manufacturer's instructions. Slides were then mounted in fluorescence anti-fading mounting medium (Vectashield, CA). In negative control experiments, the incubation with primary antibodies was

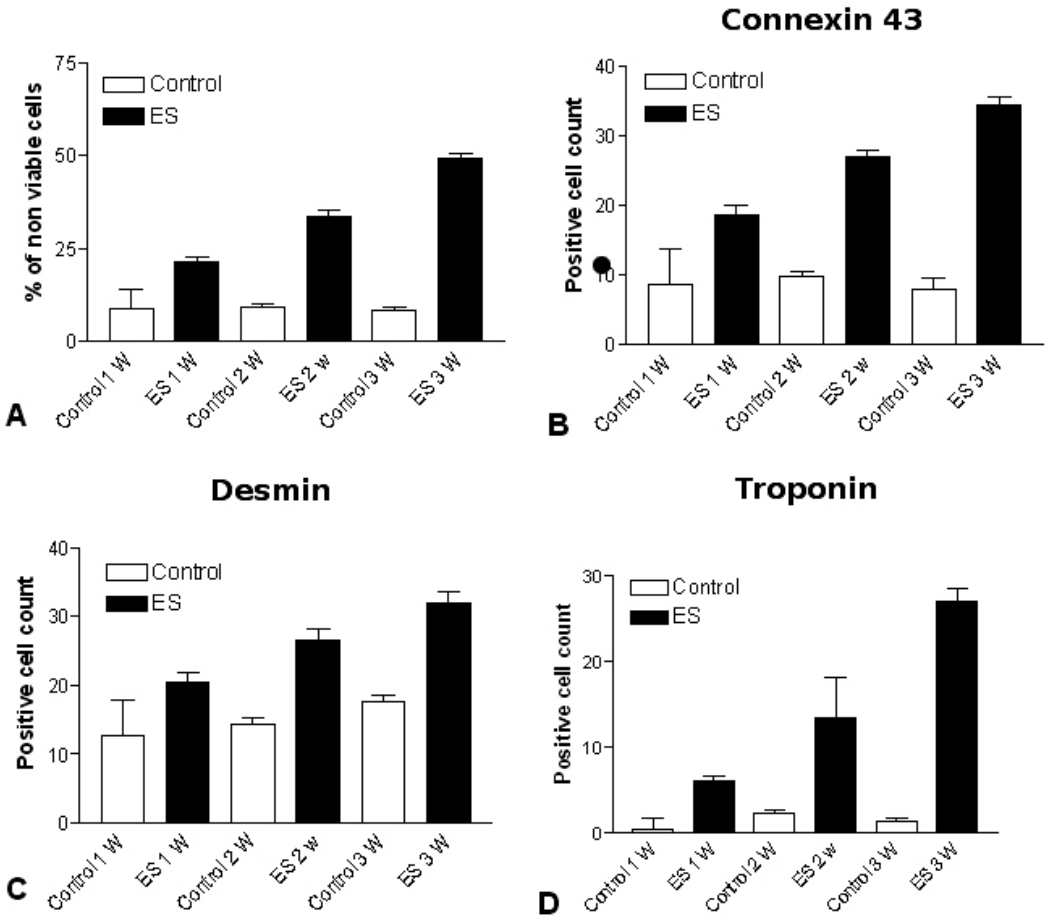

Figure 1. A) Percentage of non-viable cells following ES at different time points. Number of cells expressing Cx43 (B), Desmin (C) and cTnI (D). ES induced a significant reduction in cell viability in relation with duration of stimulation delivery (P<.001). A significant effect on cardiac marker cell expression could be detected after ES (P<.001).

omitted. Slides were viewed, with their identities masked, by fluorescence (Leica CMR) and confocal (Leica TCS-SPE) microscopy by two independent observers. Immunocytochemistry was performed on fresh cultured cell specimens and processed as previously described. (13) Briefly, cells were fixed and incubated with mouse anti-cTnI (at a dilution of 1:1000), anti-sarcomeric actin (1:1000), anti-Cx43 (1:500) anti-desmin (1:2000) primary antibodies (Sigma, MO) at 37 C. Horseradish peroxidise conjugated secondary antibodies and 3-3'-diaminobenzidine were used to detect antibody binding. Slides were viewed, with their identities masked, under a light microscope (Nikon F100) and positive cells were counted by two independent observers.

3.4. Western blotting

Both stimulated and control cells were recovered and processed for western blotting analysis as previously described (24). cTnI (Santa Cruz Biotechnology , CA), and CD29 (Neomarkers, CA) antibodies were used. Densitometry analysis was performed using Image-Pro Vers. 6.0 (Media Cybernetics, Inc. Bethesda, MD USA).

3.5. Statistical analysis

All the experiments were performed in triplicate. Data are reported as mean ± standard deviation. Data were analyzed using SPSS release 13.0 for Windows (SPSS, IL). One-way ANOVA was performed to compare groups subjected to different treatments, followed by multiple pair-wise comparisons (Tukey test). Assumptions of normality were assessed and met. The Holm-Sidak method was used to increase the power of the analysis. Pearson's product-moment *r* coefficient was calculated to evaluate correlations. Significance was determined at the 0 .05 level.

4. RESULTS

To determine the effect of electrostimualtion on hMSCs, ES (10V) was applied in 5ms pulses with a frequency of 0.5 Hz for 1-3 weeks. ES treatment initially reduced cell number. The number of cells lost was directly correlated with the duration of the stimulation (Figure 1A). Both cell viability and cell loss were affected by ES, with a progressively stronger impact as the time of stimulation increased.

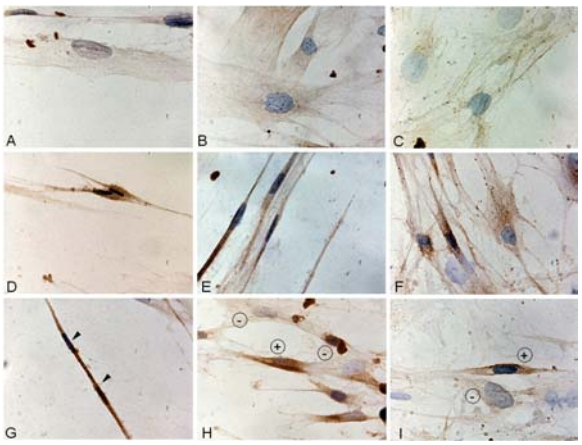

Figure 2. The effects of ES on hMSC phenotype. Immunocytochemical staining for cardiac cTnI (A,D,G), Desmin (B,E,H) and Cx43 (C,F,I) A-C) Control non-ES ; D-I) hMSCs stimulated for 3 weeks.

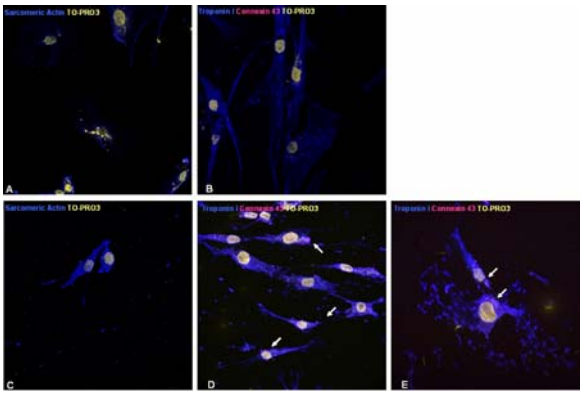

Figure 3. Analysis of cardiac markers expression by confocal microscopy. A and B) Control non-ES; C) hMSCs electrostimulated at 10V for 3 weeks. D) hMSCs electrostimulated at 10V for 2 weeks; E) hMSCs electrostimulated at 10V for 1 week. Arrows indicate membranous and perinuclear distribution of Cx43 antibody following ES.

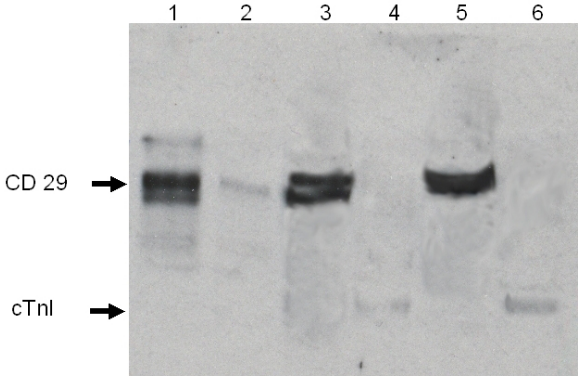

Figure 4. Western Blot analysis of cTnI and CD29 expression in hMSC cells. Lane 1: hMSC 1 week no ES; Lane 2: hMSC ES 1week 10V ; Lane 3: hMSC 2 week no ES; Lane 4: hMSC ES 2 weeks 10V; Lane 5: hMSC 3 week no ES; Lane 6: hMSC ES 3 weeks 10V.

ES induced changes in cell morphology resulting in a spindle shape, which is a feature of many myoblast primary cell cultures and cell lines (Figure 2). Cell elongation with fiber rearrangement resembling a striated-muscle-like phenotype could be detected. Moreover, following ES we could detect cell alignments and linear multinuclear fusion resembling myotubes (Figure 2 G). Interesting, cell projections developed following ES which resulted in cell-to-cell interactions not only between cardiac marker expressing cells but also between cells positive and negative for cardiac markers (Figure 2 F,H,I).

These morphological changes were accompanied by significant increases in the number of cells positive for cardiac-specific markers, cTnI, Desmin, and Cx43. Immunocytochemical analysis of the cells revealed a time-dependent increase in cTnI expression in the treated group (Figure 1 D). Similar increases could be detected in the number of cells expressing Desmin and Cx43 (Figure 1 B, C). Additionally, hMSCs electrostimulated for a longer period of time showed a significantly better arrangement of troponin and desmin inside the cytoplasm, mimicking a fibre structure indicative of a high degree of differentiation. This phenotype occurred in a larger percentage of the cells as the time of stimulation increased and the cells treated with ES for 3 weeks possessed a cellular arrangement that closely resembled that of mature myocytes (Figure 2 E and G).

In the ES-treated cells, Cx43 showed a membrane distribution (Figure 2 F, I). Next, we performed a detailed analysis of the distribution of Cx43 and cardiac-specific markers together with the changes in cell morphology using confocal microscopy (Figure 3). Cx43 was expressed in a granular pattern with both a typical membranous intercellular distribution, discussed above, and a perinuclear distribution (Figure 3 D, E). Using monesin, venom able to block molecular intracellular trafficking, and an anti-Golgi antibody, we confirmed the perinuclear Cx43 was localized to the Golgi compartment (data not shown). cTnI was co-expressed with Cx43 (Figure 3 D, E). Following ES, reorganization of cytoplasmic actin and a general elongation of the cells could be clearly visualized via confocal microscopy (Figure 3C-E and data not shown). Additionally, expression of sarcomeric actin was found to be notably increased in comparison to the control group (Figure 3 C).

We further evaluated the differentiation process induced by ES by assessing the expression of cardiac and MSC markers by western blot. An increase over time of cTnI expression was coupled with a progressive decrease in the expression of CD29, a mesenchymal stem cell marker (Figure 4).

5. DISCUSSION

Because hMSCs are attractive candidates for myocardial repair, we examined whether ES could drive these cells toward a myocardial phenotype. We found that delivery of electrical stimuli was able to induce a pre-differentiation process in hMSCs, reflected at both the

morphological and biochemical levels and resulting in a shift toward a striated muscle cell phenotype.

Stem-cell therapy for ischemic cardiac disease and secondary heart failure has the potential to become a powerful tool to reduce the morbidity and mortality of affected patients. Bone marrow-derived stem cells have a high plasticity to form cardiac myocyctes and vascular structures (25). These findings have fueled an increasing scientific interest in the use of hMSCs for myocardial repair, especially in light of their low immunogenic capacities (15, 26) and the successful initial results of clinical trials demonstrating improved ventricular function when hMSCs were transplanted to treat myocardial infarctions (27). Despite the exciting preliminary results obtained with these approaches, the necessary use of demethylating agents, growth factors or viral vectors for cardiomyocyte differentiation of hMSCs prior to injection, limits their clinical applicability. Thus, we aimed to circumvent the use of cytokines and tested the hypothesis that delivering electrical stimuli via pacing may induce hMSCs commitment towards a cardiomyocyte phenotype in a growth factor-free system.

The basis of this approach was the observation that ES prevents immobilization-induced muscle atrophy by minimizing reduction of muscle fiber cross-sectional area, interstitial fibrosis, and impairment of the blood supply, due to improvements in capillary density and the capillary-fiber ratio (28). Mimicking physiological stimulation conditions provides the correct sequence of signals to maintain and improve muscle structure and function, thus we and others have hypothesized that electrical stimuli could result in a biological shift of adult stem cells toward the muscle phenotype (29). Simulation of the cardiac cell environment via pacing and long-term ES mimics the electrical features of a beating heart and could provide an additional stimulus to direct the cellular apparatus of hMSCs toward a cardiomyocytic configuration. Jiang and colleagues showed that after introducing MSCs to treat myocardial ischemia, differentiation of these MSCs to cardiomyocytes, smooth muscle cells, and endothelial cells was time dependent and occurred 1 to 4 weeks after transplantation (30). Therefore, we extended the length of ES exposure from that previously used in our studies on fibroblasts (13). Our results here suggest that prolonging the exposure to a cardiac-like pacing stimulation pattern *in vitro* will provides adequate differentiation of hMSCs to facilitate further implantation into the heart. These partially differentiated, intermediate phenotype cell express some of the key markers of a functionally mature cardiomyocyte but will likely maintain the proliferative capacity to regenerate and compensate for myocardial loss. This partially differentiated phenotype might allow a gradual, ongoing differentiation within the cardiac environment providing time for both myocardial regeneration and electro-mechanical integration.

We observed the development of cell projections and cell-to-cell interactions not only between cardiac marker expressing cells, but even between cells positive and negative for cardiac markers. The membrane distribution of Cx43 further suggested the creation of new intercellular junctions. These interactions resembled the cell contact associated with fusion of cardiomyocytes with surrounding non-cardiomyocytes in co-cultures (31). Understanding the significance of these cell-to-cell interactions between pre-differentiated and non-differentiated MSCs requires further investigation. The possibility of a fusion phenomenon associated with ES and a cascade of cell differentiation resulting from this fusiogenic activity, although very speculative, demands future effort too.

Induction of cTnI following ES was associated with a contemporaneous decrease in the expression of the MSC marker CD29 (25) confirming an ongoing differentiation process. In light of the previous reports of myocardial microinfarcts following intracoronary injection of hMSC (32), the observed decrease in expression of this integrin superfamily member, which is involved in cell adhesion and platelet activation (33), is interesting and may address this potential clinical limitation.

Interestingly, in our previous study, ES induced similar morphological changes and cardiac-specific marker expression in two fibroblast cell lines using a shorter stimulation time but those changes were coupled with dramatic decreases in cell number and viability, supporting the idea that ES induces cell death or inhibits cell growth perhaps as part of the differentiation process. The cell population was reduced by as much as 50% in the first 3 hours, probably as the result of a necrotic process, and then stabilized after 12 hours (13). A selected ES-resistant fibroblast population may drop out of the cell cycle and later acquire myocardiocyte characteristics. Here, we stimulated the hMSCs for longer period of time and obtained similar amounts of pre-differentiated cells with a significantly lower percentage of non-viable cells within the first two weeks of treatment in comparison with the previous study. Therefore, this ES protocol may be safer and equally as effective and hMSCs may undergo similar ES-driven selection process. Whether this prolonged stimulation would also allow for a more stable phenotype induction is intriguing and surely deserves our future efforts, as do the mechanisms underlying the survival of the selected cells.

In conclusion, we demonstrated that ES is able to induce both morphological and biochemical changes in hMSCs resulting in a shift toward a striated muscle cell phenotype expressing cardiac specific markers. Remarkably, this differentiation could be achieved without any additional treatment or media supplementation or co-culturing conditions defining a very “clean” method to induce cell differentiation. These MSC-derived, partially differentiated cardiac cells may be advantageous as they may allow a gradual ongoing differentiation within the cardiac environment providing time for electromechanical integration, improving treatment efficiency and resulting in a convenient therapeutic cell source for clinical applications.

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7. REFERENCES

1. J. M. Leferovich, K. Bedelbaeva, S. Samulewicz, X. M. Zhang, D. Zwas, E. B. Lankford and E. Heber-Katz: Heart regeneration in adult MRL mice. *Proc Natl Acad Sci U S A*, 9817), 9830-5 (2001)

2. S. Itescu, M. D. Schuster and A. A. Kocher: New directions in strategies using cell therapy for heart disease. *J Mol Med*, 815), 288-96 (2003)

3. E. M. Jolicoeur, C. B. Granger, J. L. Fakunding, S. C. Mockrin, S. M. Grant, S. G. Ellis, R. D. Weisel and M. A. Goodell: Bringing cardiovascular cell-based therapy to clinical application: perspectives based on a National Heart, Lung, and Blood Institute Cell Therapy Working Group meeting. *Am Heart J*, 1535), 732-42 (2007)

4. V. F. Segers and R. T. Lee: Stem-cell therapy for cardiac disease. *Nature*, 4517181), 937-42 (2008)

5. A. A. Hagege, J. P. Marolleau, J. T. Vilquin, A. Alheritiere, S. Peyrard, D. Duboc, E. Abergel, E. Messas, E. Mousseaux, K. Schwartz, M. Desnos and P. Menasche: Skeletal myoblast transplantation in ischemic heart failure: long-term follow-up of the first phase I cohort of patients. *Circulation*, 1141 Suppl), I108-13 (2006)

6. P. C. Smits: Myocardial repair with autologous skeletal myoblasts: a review of the clinical studies and problems. *Minerva Cardioangiol*, 526), 525-35 (2004)

7. Y. Iijima, T. Nagai, M. Mizukami, K. Matsuura, T. Ogura, H. Wada, H. Toko, H. Akazawa, H. Takano, H. Nakaya and I. Komuro: Beating is necessary for transdifferentiation of skeletal muscle-derived cells into cardiomyocytes. *FASEB J*, 1710), 1361-3 (2003)

8. P. Menasche: Stem cells for clinical use in cardiovascular medicine: current limitations and future perspectives. *Thromb Haemost*, 944), 697-701 (2005)

9. C. Badorff, R. P. Brandes, R. Popp, S. Rupp, C. Urbich, A. Aicher, I. Fleming, R. Busse, A. M. Zeiher and S. Dimmeler: Transdifferentiation of blood-derived human adult endothelial progenitor cells into functionally active cardiomyocytes. *Circulation*, 1077), 1024-32 (2003)

10. S. Rangappa, J. W. Entwistle, A. S. Wechsler and J. Y. Kresh: Cardiomyocyte-mediated contact programs human mesenchymal stem cells to express cardiogenic phenotype. *J Thorac Cardiovasc Surg*, 1261), 124-32 (2003)

11. S. Rangappa, C. Fen, E. H. Lee, A. Bongso and E. K. Sim: Transformation of adult mesenchymal stem cells isolated from the fatty tissue into cardiomyocytes. *Ann Thorac Surg*, 753), 775-9 (2003)

12. M. Miura, Y. Miura, H. M. Padilla-Nash, A. A. Molinolo, B. Fu, V. Patel, B. M. Seo, W. Sonoyama, J. J. Zheng, C. C. Baker, W. Chen, T. Ried and S. Shi: Accumulated chromosomal instability in murine bone marrow mesenchymal stem cells leads to malignant transformation. *Stem Cells*, 244), 1095-103 (2006)

13. J. A. Genovese, C. Spadaccio, J. Langer, J. Habe, J. Jackson and A. N. Patel: Electrostimulation induces cardiomyocyte predifferentiation of fibroblasts. *Biochem Biophys Res Commun*, 3703), 450-5 (2008)

14. J. F. Liu, B. W. Wang, H. F. Hung, H. Chang and K. G. Shyu: Human mesenchymal stem cells improve myocardial performance in a splenectomized rat model of chronic myocardial infarction. *J Formos Med Assoc*, 1072), 165-74 (2008)

15. R. R. Makkar, M. J. Price, M. Lill, M. Frantzen, K. Takizawa, T. Kleisli, J. Zheng, S. Kar, R. McClelan, T. Miyamota, J. Bick-Forrester, M. C. Fishbein, P. K. Shah, J. S. Forrester, B. Sharifi, P. S. Chen and M. Qayyum: Intramyocardial injection of allogenic bone marrow-derived mesenchymal stem cells without immunosuppression preserves cardiac function in a porcine model of myocardial infarction. *J Cardiovasc Pharmacol Ther*, 104), 225-33 (2005)

16. K. H. Schuleri, L. C. Amado, A. J. Boyle, M. Centola, A. P. Saliaris, M. R. Gutman, K. E. Hatzistergos, B. N. Oskouei, J. M. Zimmet, R. G. Young, A. W. Heldman, A. C. Lardo and J. M. Hare: Early improvement in cardiac tissue perfusion due to mesenchymal stem cells. *Am J Physiol Heart Circ Physiol*, 2945), H2002-11 (2008)

17. G. A. Ramos and J. M. Hare: Cardiac cell-based therapy: cell types and mechanisms of actions. *Cell Transplant*, 169), 951-61 (2007)

18. J. C. Chachques, V. Mitz, M. Hero, P. Arhan, P. Gallix, F. Fontaliran and R. Vilain: Experimental cardioplasty using the latissimus dorsi muscle flap. *J Cardiovasc Surg (Torino)*, 265), 457-62 (1985)

19. J. Y. Hahn, H. J. Cho, H. J. Kang, T. S. Kim, M. H. Kim, J. H. Chung, J. W. Bae, B. H. Oh, Y. B. Park and H. S. Kim: Pre-treatment of mesenchymal stem cells with a combination of growth factors enhances gap junction formation, cytoprotective effect on cardiomyocytes, and therapeutic efficacy for myocardial infarction. *J Am Coll Cardiol*, 519), 933-43 (2008)

20. W. R. Mills, N. Mal, M. J. Kiedrowski, R. Unger, F. Forudi, Z. B. Popovic, M. S. Penn and K. R. Laurita: Stem

cell therapy enhances electrical viability in myocardial infarction. *J Mol Cell Cardiol*, 422), 304-14 (2007)

21. E. I. Cabrera Fischer, J. C. Chachques, A. I. Christen, M. R. Risk and A. Carpentier: Hemodynamic effects of cardiomyoplasty in an experimental model of acute heart failure and atrial fibrillation. *Artif Organs*, 2011), 1215-9 (1996)

22. K. Okamoto, S. Miyoshi, M. Toyoda, N. Hida, Y. Ikegami, H. Makino, N. Nishiyama, H. Tsuji, C. H. Cui, K. Segawa, T. Uyama, D. Kami, K. Miyado, H. Asada, K. Matsumoto, H. Saito, Y. Yoshimura, S. Ogawa, R. Aeba, R. Yozu and A. Umezawa: 'Working' cardiomyocytes exhibiting plateau action potentials from human placenta-derived extraembryonic mesodermal cells. *Exp Cell Res*, 31312), 2550-62 (2007)

23. C. Rucker-Martin, F. Pecker, D. Godreau and S. N. Hatem: Dedifferentiation of atrial myocytes during atrial fibrillation: role of fibroblast proliferation *in vitro*. *Cardiovasc Res*, 551), 38-52 (2002)

24. L. Ding, X. Liang, D. Zhu and Y. Lou: Peroxisome proliferator-activated receptor alpha is involved in cardiomyocyte differentiation of murine embryonic stem cells *in vitro*. *Cell Biol Int*, 319), 1002-9 (2007)

25. M. F. Pittenger, A. M. Mackay, S. C. Beck, R. K. Jaiswal, R. Douglas, J. D. Mosca, M. A. Moorman, D. W. Simonetti, S. Craig and D. R. Marshak: Multilineage potential of adult human mesenchymal stem cells. *Science*, 2845411), 143-7 (1999)

26. K. Le Blanc, C. Tammik, K. Rosendahl, E. Zetterberg and O. Ringden: HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp Hematol*, 3110), 890-6 (2003)

27. S. L. Chen, W. W. Fang, F. Ye, Y. H. Liu, J. Qian, S. J. Shan, J. J. Zhang, R. Z. Chunhua, L. M. Liao, S. Lin and J. P. Sun: Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. *Am J Cardiol*, 941), 92-5 (2004)

28. L. Qin, H. J. Appell, K. M. Chan and N. Maffulli: Electrical stimulation prevents immobilization atrophy in skeletal muscle of rabbits. *Arch Phys Med Rehabil*, 785), 512-7 (1997)

29. Y. Kawahara, K. Yamaoka, M. Iwata, M. Fujimura, T. Kajiume, T. Magaki, M. Takeda, T. Ide, K. Kataoka, M. Asashima and L. Yuge: Novel electrical stimulation sets the cultured myoblast contractile function to 'on'. *Pathobiology*, 736), 288-94 (2006)

30. W. Jiang, A. Ma, T. Wang, K. Han, Y. Liu, Y. Zhang, A. Dong, Y. Du, X. Huang, J. Wang, X. Lei and X. Zheng: Homing and differentiation of mesenchymal stem cells delivered intravenously to ischemic myocardium *in vivo*: a time-series study. *Pflugers Arch*, 4531), 43-52 (2006)

31. K. Matsuura, H. Wada, T. Nagai, Y. Iijima, T. Minamino, M. Sano, H. Akazawa, J. D. Molkentin, H. Kasanuki and I. Komuro: Cardiomyocytes fuse with surrounding noncardiomyocytes and reenter the cell cycle. *J Cell Biol*, 1672), 351-63 (2004)

32. P. R. Vulliet, M. Greeley, S. M. Halloran, K. A. MacDonald and M. D. Kittleson: Intra-coronary arterial injection of mesenchymal stromal cells and microinfarction in dogs. *Lancet*, 3639411), 783-4 (2004)

33. O. Pradier, M. Surquin, P. Stordeur, L. De Pauw, P. Kinnaert, P. Vereerstraeten, P. Capel, M. Goldman and D. Abramowicz: Monocyte procoagulant activity induced by *in vivo* administration of the OKT3 monoclonal antibody. *Blood*, 879), 3768-74 (1996)

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