34th Annual International Conference of the IEEE EMBS
San Diego, California USA, 28 August - 1 September, 2012

Influence of electrical stimulation on 3D-cultures of Adipose Tissue Derived Progenitor Cells (ATDPCs) behavior

C. Castells-Sala[1*], B. Sanchez[2*], L. Recha-Sancho[1], V. Puig[1], R. Bragos[2] and C. E. Semino[1]

Abstract— **Tissue engineering has a fundamental role in regenerative medicine. Still today, the major motivation for cardiac regeneration is to design a platform that enables the complete tissue structure and physiological function regeneration of injured myocardium areas. Although tissue engineering approaches have been generally developed for two-dimensional (2D) culture systems, three-dimensional (3D) systems are being spotlighted as the means to mimic better in vivo cellular conditions. This manuscript examines the influence of electrical stimulation on 3D cultures of adipose tissue-derived progenitor cells (ATDPCs). ATDPCs cells were encapsulated into a self-assembling peptide nanoscaffold (RAD16-I) and continuously electro stimulated during 14-20 days with 2-ms pulses of 50mV/cm at a frequency of 1 Hz. Good cellular network formation and construct diameter reduction was observed in electro stimulated samples. Importantly, the process of electro stimulation does not disrupt cell viability or connectivity. As a future outlook, differentiation studies to cardiomyocytes-like cells will be performed analyzing gene profile and protein expression.**

I. Introduction

Heart failure is the end-stage of many cardiovascular diseases, but the main problem is the presence of necrotic tissue due to an acute myocardial infarction which deeply affects heart function [1]. As myocardial has very limited regeneration capacity, new therapeutic approaches to recover this function are highly required. Current treatments under development are looking for effective and less invasive treatments, hence therapies based on stem cells open a new world of possibilities since they can divide and differentiate into different specialized cell types [2]. One of the major concerns about cellular therapy is which cellular type is the more appropriate for cardiac regeneration [3], [4]. Adult stem cells are potentially ideal candidates for tissue engineering as they are currently believed to be less likely to initiate rejection after transplantations as a result of patients own cells could be expanded in culture, inducted to differentiate and then reintroduced into the patient. Until recently, bone marrow have been the major tissue source of adult stem cells for both experimental and clinical studies, but recent studies support the existence of stem cells in adipose tissues. Adipose tissue-derived stem cells (ASCs) are very similar to bone marrow stem cells (BMSCs) and several reports proved their potential to differentiate into various cell types when cultured in defined medium *in vitro*, even excitable cells such as cardiomyocytes and neurons [5]. Additionally it has been shown that they provide clear advantage over other stem cell sources due to the ease of accessibility, isolation and expansion [6].

In cell-based therapy, some authors have been shown beneficial effects when cells are injected directly into injured heart [7], [8]. However, control the location of grafted cells after transplantation is difficult, since myocardium is a highly vascularized tissue. For this reason, cell encapsulation into natural or artificial scaffolds (collagens, polymeric fibers, respectively) could be a better option to maintain the cells in the infarcted zone and/or pre-train them*in vitro* to stand the mechanical forces of the host tissue [9], [10], [3], [11].

We have evaluated the influence of a soft nanofiber scaffold (RAD16-I self-assembling peptide scaffold which reproduce a 3D environment) on adipose tissue-derived progenitor cells (ATDPCs). RAD16-I is a hydrogel that self-assemble in the presence of monovalent cations ending in the development of a nanofiber network with a mesh size of 5-200nm in diameter and over 99

Transplanted cells may contribute to different events which can take part in the improvement of heart function, such as differentiation into cardiomyocytes, angiogenesis, cell fusion or paracrine effects. In this case in particular, successful cell transplantation should take into account electrical and mechanical coupling with the host myocardium[12]. The engineered cardiac construct should connect to the electrical synctium of the existing myocardium, rather than having spontaneous contractile activity.

A hallmark of functional myocardium is its ability to propagate electrical impulses and to respond to these impulses by synchronized contractions that generate forces for pumping blood. The cells forming the native heart form a 3D synctium which allows propagation of the action potentials that regulate synchronous contraction signals across specialized intercellular junctions called gap junctions [13]. This stimulation spreated throughout the heart cause depolarization of cellular membranes and activation of the contractile apparatus. So it is believed that electrical stimulation to cardiac constructs markedly enhanced the

This work was supported in part by grants from the Spanish Ministry of Science and Innovation, Redes de Investigacion del Instituto de Salud Carlos III (REDINSCOR, RD06/0003), Fondo Europeo de Desarollo Regional (FEDER), the Spanish Ministry MICINN SAF2008-05144-C02-02 and SAF2011-30067-C02-02, 080331/2 from Fundacio La Marato de TV3 and RECATABI European project (Grant agreement no.: CP-FP 229239-2 RECATABI).

C. Castells-Sala, L. Recha-Sancho, V. Puig and C.E. Semino are with the Tissue Engineering Laboratory, Bioengineering Department, Institut Quimic de Sarria, Universidad Ramon Llull, Barcelona, SPAIN. cristina.castells@iqs.url.edu

B. Sanchez and R. Bragos are with Electronic and Biomedical Instrumentation Group, Departament Enginyeria Electronica, Universitat Politecnica de Catalunya (UPC), Barcelona, 08034, SPAIN. benjamin.sanchez@upc.edu

* Both authors contributed equally to this work.

contractile behavior [14]. Moreover, studies developed by Milica Radsic et.al. show that electrical field stimulation induce remarkable enhancement cell alignment of neonatal rat ventricular myocytes seeded onto Matrigel, synchronous construct contraction and ultrastructural organization over only 8 days of cultivation [15], [16].

We propose that apply electrical signals to this 3D constructs mimicking native heart will induce phenotypic changes in ATDPCs resulting in the pre-commitment of these cells to a myocardial fate, so it is needed a setup to electro stimulate cells encapsulated in RAD16-I. Here is reported a novel system to electro stimulate biomimetic approach provided by a hydrogel in which ATDPCs were cultured *in vitro*.

II. Materials and Methods

In this section we briefly explain the materials and methods strategy carried out during the experimental work. First, the electrical stimulation setup is described in Section II-A. Next, Sections II-B, II-C and II-D describe the preparation of the biological samples. Finally, Section II-E details the electrical stimulation protocol tested.

A. Electrical Stimulation Setup

The stimulation unit setup used was custom made and consisted of a combination of a monophasic programmable electrical device, a printed circuit board (PCB) (FR-4 plastic laminate designed with Ultiboard Circuit Design, National Instruments) that enabled electrical stimulation of up to six petri dishes (10x35 mm), and two Platinum-Iridium wire electrodes (0.5 mm diameter) (Figure 1) per culture plate and insert (0.4 m pore, 12 mm external diameter, 9mm internal diameter Millipore, cat PICM01250) (see Figure 1). After the

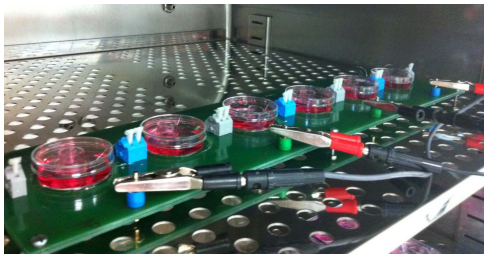

Fig. 1. Detail of the electrical stimulation setup inside the cell culture incubator: the Printed Circuit Board (PCB), cultures plates and inserts including the stimulation electrodes. See text for details.

mechanization of the inserts with the electrodes, both were sterilized by gamma ray (25 units are necessary to get rid of contaminating spores). Then, once set in the petri dishes, they were treated with ethanol and ultraviolet light before start cell culture.

For a given electric field amplitude applied to the cells using two stimulation electrodes, a fraction of the applied electric field is lost near the electrodes due to the electrode impedance resulting from the medium-electrode interface. As a result, the effective electric field applied to the cells is variable, depending on the electrode material, the electrodes, effective stimulation area and the medium volume. To mitigate this effect, the electrodes were twisted at the end. Furthermore, the experimental protocol conditions were modified according to the measured effective electric field applied to the cells until it was verified within a set of experimental validation measurements that effective electrical applied field to the cultured cells was 50mV/cm.

On the other end, each of the electrodes was connected to the connector provided by the PCB and to the electrical stimulator through an isolator stage.

As for the electrical connection between the six corresponding culture plates, three of the culture plates were connected in parallel and then in series with the other three also connected in parallel. With this electrical connection, a sample could be removed without therefore having to stop the experiment.

B. Cell Culture

Adipose tissue derived progenitor cells (ATDPCs) from different patients were provided by Dr. A.Bayes-Genis from ICREC Research Program (Hospital Universitari Germans Trias i Pujol). The cells were cultured in 75 cm^2 flasks in α-MEM media supplemented with 10 % (v/v) fetal bovine serum (FBS; Lonza), 1 % P/S and 1 % glutamine sterilized filtered to eliminate possible solids formed. Cells from between 4 and 6 patients were mixed to obtain a pool of cells which was cultured until passage 8. The cells were trypsinized once per week to expand them and the medium was changed every 2 days.

C. Cell Encapsulation

For experiments in three-dimensional (3D) milieu, commercial 1% RAD16-I (Puramatrix, Becton & Dickinson) was used. It consist of a sequence of 16 aminoacids ((Arg-Ala-Asp-Ala)$_4$) 1% (w/v) in water. ATDPCs ((9 passage) were trypsinized from the culture flasks, counted and resuspended in 10% sucrose at a final concentration of $4 \cdot 10^6$ cells/mL. Parallel, Puramatrix was diluted with sucrose to obtain a solution of 0,3% (w/v) RAD16-I in sucrose 10 %. Then, equal volume of cell solution and RAD16-I 0.3% (w/v) solution were mixed to render a final cell suspension of $2 \cdot 10^6$ cells/mL, 10 % sucrose. This suspension was mixed carefully and 80 μL were loaded into a 9-mm diameter cell culture inserts (PICM012050, Millipore) previously placed in a 6-well plate and equilibrated with 500 μL of media outside the insert. Once the mixture was loaded, the medium wets the membrane underneath the insert and induces the self-assembly of RAD16-I due to the increase of the ionic strength. Therefore, the peptide form a gel with the cells embedded in the scaffold. After gel formation, some washes with culture medium were performed to wash out the remaining sucrose and finally 0.5 mL of media were loaded in the insert and 2.5 mL in the well outside the insert. Incubation was performed at 37 C with 5 % carbon dioxide. The medium was changed every 2 days.

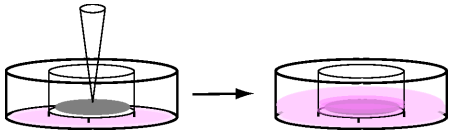

Fig. 2. Scheme of the cell seeding. See text for details.

D. Live and Dead

The LIVE/DEAD Viability/Cytotoxicity Kit for mammalian cells (Invitrogen cat-no.: L-3224) was used to prove cell viability in the hydrogel. The results were in a fluorescent microscope (Zeiss).

E. Electrical Stimulation Protocol

With the aim of electro stimulate cells cultured growing in a three dimensional milieu, ATDPCs were encapsulated in inserts with platinum electrodes connected to the stimulator (1 construct per insert and 2 inserts per Petri dish) as described in materials. Cell encapsulation was performed as explained but Petri dishes were filled with 3 ml of culture medium instead of 2.5mL. Constructs were pre-cultured without electrical stimulation during 7 days to allow cells to adapt to the new milieu. At day 7, all the medium in the inserts was removed an 80 μL of cell culture agarose 0.75 % was loaded on the construct. The gelification of the agarose fix the construct in the insert assuring that it will not change the position and the electrical field will always affect in the same direction. Once the constructs were fixed, inserts and Petri dishes were filled with the appropriate media. Trains of electrical pulses (rectangular, 2 ms, 50 mV/cm) which are similar of native myocardium were applied continuously for an additional 14 days. Constructs cultured without electro stimulation under identical conditions served as non-stimulated controls.

III. Experimental results

This section describes the preliminary experimental results from stimulating ATDPCs within a 3D environment. First, Sections III-A and III-B evaluate at a cellular level the cell network viability. Finally, the 3D construct diameter reduction is analyzed at several days during the electrical stimulation.

A. Cell Network

After the first hours of encapsulation in 3D scaffolds, cells started to recover from trypsinization procedure. Initially, they exhibit a rounded shape that was transformed to an elongated appearance over days of culture. During this process cells elongated and spreaded supporting intercellular connections and network formation. To follow the network formation over time Dapi and Phalloidin staining was performed. Thus, Dapi stains nuclei and Phalloidin stains actin filaments of the cytoskeleton obtaining the results shown in Figure3. Network formation of ATDPCs increased progressively along the time and cells appeared spread and inter-connected after 7 days of 3D culture.

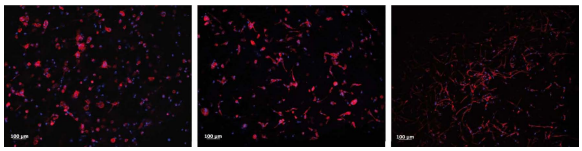

Fig. 3. Dapi and phalloidin staining of ATDPCs growing in RAD16-I at day 1 (left), 4 (middle) and 7 (right). Dapi (blue) targets DNA in the cell nucleus (ultraviolet excitation with blue emission) and phalloidin (red) the cytoskeletal filamentous actin network.

B. Live and Dead

To asses that electrical stimulation does not affect the viability of the cells growing in this three dimensional milieu, Live and Dead assay was performed. As it can be observed in the Figure 4, most of the cells are alive whereas few of them are dead. Comparing with non-electrical stimulated constructs the same pattern was observed (data not shown), so it can be concluded that electrical stimulation do not affect the viability.

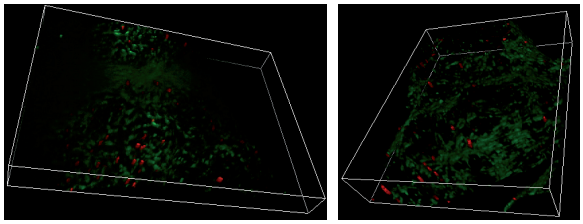

Fig. 4. Live (green) and dead (red) assay of ATDPCs growing in a self-assembling peptide nanoscafold RAD16-I, after 14 days of culture (left) and after 20 days of culture (right). Good viability can be observed in these 3D cultures.

C. Construct Diameter Reduction

Over the days, the dense network formed promoted the contraction of the construct. This was possible due to the nature of the peptide RAD16-I, as the non-covalent interactions between the nanometric fibers allow the cells to interact and migrate. Consequently, there was a correlation between the development of a cellular network and the contraction of the structure.

Once the cell network was correctly formed constructs were cultured with the application of electrical stimulation. The effect of electrical stimulation in construct diameter reduction was analyzed at 14 days of culture (see Figure 5). It can be observed that although the electrical stimulation induces a faster diameter reduction, after 7 days the contraction is fast the same for samples electrical stimulated and control samples. This could indicate that electrical stimulation accelerated the diameter reduction which can be related with the improvement of cell interaction and connectivity

IV. Discussion

Until recently most experiments in this area have been developed in 2D cultures but as it has been described above this kind of culture does not mimic as well as possible the characteristics of *in vivo* cells growth. We initially examined

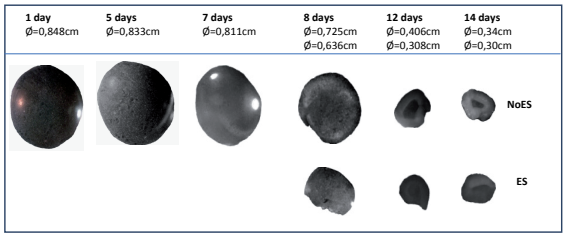

Fig. 5. Evolution of the 3D constructs diameter reduction at several days.

these cells in self-assembling peptide gels RAD-16 I studying their viability, network formation and diameter reduction within a 3D environment. Right after encapsulation, the cells have a round shape, but after a couple of days in culture they start to stretch and to develop contacts between them and the peptide nanofibers. After one week, a network can be observed and the whole construct starts to diminish the diameter due to the cellular stretching. Once the cells are comfortable in the new environment electrical induction was performed observing that the reported conditions for electrical stimulations were not harmful for cells survival: no differences in the viability of cells compared with samples no-electro stimulated were observed, which is in accordance with previous studies [17]. Additionally no differences were observed between non- and electro stimulated constructs referring to diameter reduction.

While the role of electrical stimulation has been widely investigated in neonatal rat cardiac myocytes much less attention has been paid to the differentiation process when considering adult stem cells. In this manuscript, we evaluated the potential of 3D constructs from adipose-derived adult stem cell source for future applications in cardiac tissue engineering and cell therapies.

V. Conclusions

The results which have been presented in this work suggest that three-dimensional environment provided by the self-assembling peptide nanoscaffold RAD16-I may be a good milieu to enhance the differentiation of adipose-derived stem cells into cardiac-like cells. Moreover, the effect of electrical stimulation makes of this scaffold a promising material to train cells using this kind of stimulus. It is important to mention that important areas for future work may include the analysis of gene profile by PCR and protein expression Western Blotting. Additionally electrical stimulation and culture media conditions may be optimized to improve cellular differentiation into cardiac lineage.

VI. Acknowledgements

The authors would like to thank Dr. A.Bayes-Genis and Dr. C. Soler from ICREC Research Program, Hospital Universitari Germans Trias i Pujol for providing us the ATDPCs cells to carry out the experimental part.

References

[1] P. Anversa, J. Kajstura, and A. Leri, *Cellular Basis for Myocardial Repair and Regeneration*, second edi ed. Elsevier Inc.

[2] J. Leor, Y. Amsalem, and S. Cohen, "Cells, scaffolds, and molecules for myocardial tissue engineering." *Pharmacology & therapeutics*, vol. 105, no. 2, pp. 151–63, Feb. 2005.

[3] M. Cortes-Morichetti, G. Frati, O. Schussler, J.-P. D. Van Huyen, E. Lauret, J. a. Genovese, A. F. Carpentier, and J. C. Chachques, "Association between a cell-seeded collagen matrix and cellular cardiomyoplasty for myocardial support and regeneration." *Tissue engineering*, vol. 13, no. 11, pp. 2681–7, Nov. 2007.

[4] C. Gonzales and T. Pedrazzini, "Progenitor cell therapy for heart disease." *Experimental cell research*, vol. 315, no. 18, pp. 3077–85, Nov. 2009.

[5] X. Bai, J. Ma, Z. Pan, Y.-H. Song, S. Freyberg, Y. Yan, D. Vykoukal, and E. Alt, "Electrophysiological properties of human adipose tissue-derived stem cells." *American journal of physiology. Cell physiology*, vol. 293, no. 5, pp. C1539–50, Nov. 2007.

[6] M. Tobita, "Adipose-derived Stem Cells: Current Findings and Future Perspectives," *Discovery Medicine*, vol. 057, no. 11, pp. 160–170, 2011.

[7] J. D. Dowell, M. Rubart, K. B. S. Pasumarthi, M. H. Soonpaa, and L. J. Field, "Myocyte and myogenic stem cell transplantation in the heart." *Cardiovascular research*, vol. 58, no. 2, pp. 336–50, May 2003.

[8] A. Bayes-Genis, C. Soler-Botija, J. Farré, P. Sepúlveda, A. Raya, S. Roura, C. Prat-Vidal, C. Gálvez-Montón, J. A. Montero, D. Büscher, and J. C. Izpisúa Belmonte, "Human progenitor cells derived from cardiac adipose tissue ameliorate myocardial infarction in rodents." *Journal of molecular and cellular cardiology*, vol. 49, no. 5, pp. 771–80, Nov. 2010.

[9] R. L. Carrier, M. Papadaki, M. Rupnick, F. J. Schoen, N. Bursac, R. Langer, L. E. Freed, and G. Vunjak-Novakovic, "Cardiac tissue engineering: cell seeding, cultivation parameters, and tissue construct characterization." *Biotechnology and bioengineering*, vol. 64, no. 5, pp. 580–9, Sep. 1999.

[10] K. L. Christman and R. J. Lee, "Biomaterials for the treatment of myocardial infarction." *Journal of the American College of Cardiology*, vol. 48, no. 5, pp. 907–13, Sep. 2006.

[11] O. Schussler, J. C. Chachques, T. G. Mesana, E. J. Suuronen, Y. Lecarpentier, and M. Ruel, "3-Dimensional Structures To Enhance Cell Therapy and Engineer Contractile Tissue." *Asian cardiovascular & thoracic annals*, vol. 18, no. 2, pp. 188–98, Feb. 2010.

[12] M. Q. Chen, X. Xie, R. Hollis Whittington, G. T. a. Kovacs, J. C. Wu, and L. Giovangrandi, "Cardiac differentiation of embryonic stem cells with point-source electrical stimulation." *Conference of the IEEE Engineering in Medicine and Biology Society.*, vol. 2008, pp. 1729–32, Jan. 2008.

[13] S. Kanno and J. E. Saffitz, "The role of myocardial gap junctions in electrical conduction and arrhythmogenesis." *Cardiovascular pathology : the official journal of the Society for Cardiovascular Pathology*, vol. 10, no. 4, pp. 169–77, 2001.

[14] N. Tandon, C. Cannizzaro, P. H. Chao, R. Maidhof, A. Marsano, and H. T. Au, "Electrical stimulation systems for cardiac tissue engineering," *Nat Protoc*, vol. 4, no. 2, pp. 155–73, 2009.

[15] M. Radisic, H. Park, S. Gerecht, C. Cannizzaro, R. Langer, and G. Vunjak-Novakovic, "Biomimetic approach to cardiac tissue engineering." *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, vol. 362, no. 1484, pp. 1357–68, Aug. 2007.

[16] G. Vunjak-Novakovic, N. Tandon, A. Godier, R. Maidhof, A. Marsano, T. Martens, and M. Radisic, "Challenges in cardiac tissue engineering." *Tissue engineering. Part B, Reviews*, vol. 16, no. 2, pp. 169–87, Apr. 2010.

[17] M. Radisic, L. Yang, J. Boublik, R. J. Cohen, R. Langer, L. E. Freed, and G. Vunjak-Novakovic, "Medium perfusion enables engineering of compact and contractile cardiac tissue." *American journal of physiology. Heart and circulatory physiology*, vol. 286, no. 2, pp. H507–16, 2004.