IMPACT OF CARBON NANOFORMS ON HIPSC-DERIVED CARDIOMYOCYTES

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Introduction

Human induced pluripotent stem cells (hiPSCs), generated from somatic cells after overexpression of defined transcription factors (most often OCT4, SOX2, KLF4 and c-MYC) demonstrate capacity to efficiently differentiate into cardiomyocytes (hiPSCs-CM). Due to such properties, hiPSCs provided novel opportunities: i) to obtain patient-specific cardiomyocytes ii) to utilize such cells in investigating mechanisms of heart diseases and in drug testing, iii) to generate cells applicable in regenerative medicine [1]. One of the key challenges in the successful usage of hiPSCs-CM in medicine is guaranteeing their proper growth, proliferation and maturation. As current state of the knowledge regarding behaviour of stem cells and their progenies within the body suggests, this cannot be done without mimicking interactions that are naturally occurring between the cells and the extracellular matrix (ECM) [2]. Those interactions could possibly be mimicked by using carefully selected scaffolds. Materials of different morphology, stiffness, chemical composition and electrical conductivity, should be able to dictate the stem cells' fate, both *in vitro* and *in vivo* [3-5].

Due to chemical and morphological biomimetism, good biocompatibility and excellent electrical conductivity, nanoforms of carbon are currently regarded as promising materials in fabricating new generation of scaffolds for culturing various cell types [6-8]. In this preliminary study, an impact of the morphology and chemical composition of two nanoforms of carbon (NC, namely, carbon nanotubes and graphene) on the viability, proliferation and maturation of the hiPSCs-CM was evaluated. Additionally, ability of NCs to activate heme oxygenase-1 (HO-1), a major cytoprotective factor demonstrating anti-inflammatory, anti-apoptotic and pro-angiogenic properties which also regulates cardiac differentiation of pluripotent stem cells was analysed [9]. Two forms of contact were tested – NCs used as matrix additive in Geltrex ® and in the form of layers deposited on the surface of titanium. The aim was to investigate their potential applicability as scaffolds that are to be electrically stimulated in order to enhance cardiac differentiation of hiPSCs and maturation of hiPSCs-CM.

Materials and Methods

Oxidized CNTs were fabricated according to the procedure established in our previous studies [10, 11] while oxidized graphene oxide (GO) was purchased from the NanoAmor Inc. and used as-received. Both NCs were subjected to additional treatment involving modification with electrically conductive polyaniline (PANI, emeraldine salt, Sigma Aldrich) to introduce NH group into the material. Chemical composition and morphology of the NCs were tested via XPS, FTIR, SEM and TEM.

For the obtainment of the layers, electrophoretic deposition was performed on the surface of degreased, and etched in 5% HF titanium. Modification of Geltrex ® was done by mixing 2 ml of NC/DMEM/F12 suspension (0.05mg/ml for CNTs and 0.1mg/ml for GO) with 20 µl of Geltrex ®. 250 µl of each of the the as prepared mixture were then pippeted into 8 separate culture wells. Geltrex ® in DMEM/F12 were used as a reference. 4,5 x 10^4 hiPSCs were seeded on Geltrex ® (control) and CNT-modifed Geltrex ® and upon reaching confluency, cardiac differentiation was initiated according to previously established protocol [12]. Briefly, on day 0 cells were stimulated with CHIR99021 (WNT pathway activator) in RMPI medium supplement with B27 lacking insulin to induce transition into mesoderm and subsequently stimulated with IWR-1 (WNT pathway inhibitor) on day 3 to form cardiac mesoderm. Further process was performed in RMPI medium supplemented with B27. On day 20, generated cells were collected and the efficiency of differentiation was analysed using FACS-based quantification of troponin T positive cells. Additionally, 5 x 10^4 hiPSCs-CM were seeded on Geltrex ® (control) or different NC-based scaffolds, collected 24h, 48h and 72h after seeding and counted to evaluate the effect of NCs on attachment and proliferation of cells.

Results and Discussion

- Efficiency of differentiation is not impaired when the cells are cultured on the NCs-modified Geltrex ®
- When in the form of a layer, NCs were found to inhibit the cells adhesion, with stronger effect observed for the PANI – modified materials. At the same time, in all of the materials there is a trend of enhancing the cells proliferation - a stable, linear growth in the cell number is observed throughout the experiment
- There is an observable tendency of NCs not stimulating the cells' maturation and increasing the expression of the HO-1 in the tested cells, possibly by inducing the oxidative stress.

Conclusions

In this study, biofunctionality of NCs as potential scaffolds for culturing the hiPSCs-CM was evaluated. The materials were found to be non-cytotoxic. When used as Geltrex ® matrix additive, the NCs did not impair the efficiency of the cells differentiation, while in the form of the layers, the NCs were found to induce higher expression of HO-1 in cells which may be beneficial in protecting against potential pathologies developing in un-matured culture cells.

All in all, the NCs were found to be interesting materials for future cultures involving electrical stimuli to enhance the cells' differentiation and maturation.

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