GRAPHENE-BASED SUBSTRATES INFLUENCE BIOLOGICAL AND FUNCTIONAL PROPERTIES OF HUMAN UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS

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[Engineering of Biomaterials 138 (2016) 24]

Introduction

Cardiovascular diseases are one of the most frequent causes of death in developed countries [1]. Thus, regeneration of damaged cardiac tissue is leading challenge of contemporary medicine. Recently, significant efforts were placed on stimulation of reparatory mechanisms of injured myocardium, including utilization of mesenchymal stem cells (MSCs) [2]. However, as MSCs cardiomyogenic potential is limited, there are several attempts to increase their therapeutical efficacy, including utilization of biocompatible culture surfaces [3]. Recently, graphene-based biocomposites emerged as promising materials for biomedical applications [4]. However, the possibility of their utilization as surfaces for MSCs culture still requires further investigation.
Thus, the aim of current study was to evaluate the potential of graphene-oxide (GO) and reduced graphene-oxide (rGO) as a culture scaffolds for human MSCs isolated from umbilical cord Wharton's jelly (hUC-MSCs).

Materials and Methods

GO and rGO were prepared from graphite according to the Marcano method. We tested different size and thickness of graphene flakes as well as a type of utilized solvent (aqueous or ethanol), to determine the most effective graphene substrate for hUC-MSCs culture. Next, we investigated the effect of GO and rGO on the biological and functional properties of hUC-MSCs, including morphology, proliferative capacity and migratory activity of the cells. Furthermore, we employed flow cytometry to evaluate the apoptosis rate of cells stained with annexin V and viability dye 7-AAD. Finally, we performed gene expression analyses in order to test the effectiveness of cardiomyogenic differentiation of hUC-MSCs cultured on different graphene-based substrates.

Results and Discussion

Obtained results revealed that graphene-based surfaces constitute non-toxic culture substrates for hUC-MSCs, but their effect depends on the thickness of graphene layer and the level of graphene reduction. Importantly, we observed, that highly reduced rGO flakes affect cell proliferation and survival of hUC-MSCs. Moreover, microscopic analysis of cells demonstrated that graphene-based substrates may stimulate elongation of hUC-MSCs in a flake size-dependent manner. In particular, thicker and larger layers of GO flakes promoted elongated morphology of the cells. Additionally, quantitative analysis of cell trajectories demonstrated that cells cultured on GO prepared in ethanol solvent migrated faster, comparing to the control plates and aqueous GO solution. Importantly, our results shown that GO may enhance hUC-MSCs differentiation toward cardiomyocytes *in vitro*.

Conclusions

Our study provides evidence that graphene-based substrates, particularly GO, constitutes a suitable substrate for hUC-MSCs *in vitro* culture and may enforce functional properties of cells, important for their therapeutical efficacy. However, further studies are required to analyze the impact of several graphene-based materials for SCs culture and their applicability in cardiac regeneration.

Acknowledgments

This study was funded by Symfonia 3 NCN grant *"Optimization of biocompatible scaffolds combining graphene and defined stem cell populations for tissue regeneration*" (UMO-2015/16/W/NZ4/00071).

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