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

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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 potential of graphene and defined stem cell populations for tissue regeneration [3]. "Optimization of biocompatible scaffolds combining graphene and defined stem cell populations for tissue regeneration" (UMO-2015/16/W/NZ4/00071).

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 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 therapeutic efficacy. However, further studies are required to analyze the impact of several graphene-based materials for SCs culture and their applicability in cardiac regeneration.

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