5-Azacytidine mediated hMSC differentiation on nanofibrous scaffolds for skeletal muscle regeneration

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Myopathies represent a major cause of disability in the world. In 2020, an estimated 200 millions new cases of myopathies will be diagnosed. Myopathies are characterized by structural and functional alterations of muscle fibers, resulting in loss of mobility and decreased life quality (Volakliset al., 2015). Usually, myopathies are caused by inherited genetic defects (e.g., muscular dystrophies) or endocrine, inflammatory and metabolic disorders.
Currently, cell therapy represents an interesting challenge in the treatment of skeletal muscle diseases, requiring cells with the capacity to address the formation of new muscle fibres such as adult human mesenchymal stem cells (hMSCs). In particular, several strategies based on pharmacological treatments have been tested for treating symptoms of acute and chronic skeletal muscle injuries, not showing satisfactory results (Gintjee et al., 2014).
In this context, 5-Azacytidine (5-AZA) – a DNA-methylation inhibitor just known for promoting up-regulation of muscle genes and hMSC differentiation – may play a key role in the formation of the striated sarcomeres, their typical morphology and the functionality (Karalaki et al., 2009).
In this study, we aim at investigating the myogenic effect of 5-azacytidine (1 and 5 μM) on hMSCs plated onto fibres made of Polycaprolactone (PCL), in order to elucidate the contribution of 3D structure onto the biological response in the presence of 5-azacytidine at different concentration. PCL fibres were produced by electrospinning to exactly mimic morphological organization of the extracellular matrix as reported elsewhere (Guarino et al., 2011). Bone marrow hMSCs plated onto PCL nanofibers were grown in myogenic medium and treated with 5-AZA at different concentration (i.e., 1 or 5 μM) from 7 to 28 days. hMSC differentiation by YIP-1B and MYH-2 expression, skeletal muscle cells formation by Azan Mallory stain and myotubes morphology by SEM were analysed. Cell differentiation was further proved by treatment with two endogenous drugs (i.e, oxytocin, TWEAK-protein).
Here we demonstrate that 5-AZA in presence of PCL nanofibers significantly increased YIP-1B (index of myogenic differentiation) compared to plate control. SEM clearly showed changes in hMSC morphology under 5-AZA stimulation. Azan Mallory stain confirmed hMSC differentiation in skeletal muscle cells. MYH-2 expression indicated a completed differentiation of stem cells into myogenic phenotypes; in fact culture conditions using myogenic culture medium plus 5-AZA (5 μM) were able to induce myotubes formation from hMSCs after 21 days of culture. Furthermore, oxytocin and tweak protein increased YIP-1B expression in hMSCs plated onto PCL compared to YIP-1B expression in hMSCs cultured on flat surface at day 28, thus confirming the correct advance of myogenesis.
Summarizing, in the presence of PCL nanofibers, 5-AZA is able to promote skeletal muscle regeneration for lower concentration (1μM) respect to 2D culture, thus confirming the active role of 3D fibre network on influencing the *in vitro* interaction of 5-AZA with hMSC cells. Hence, the proposed study paves the way towards new models to validate the efficacy of therapeutic implantable devices for clinical experimentation.

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