A G-CSF Functionalized Scaffold for Stem Cells Seeding: a Differentiating Device for Cardiac Purposes
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Introduction
Myocardial infarction and its consequences represent one of the most demanding challenges in cell therapy and regenerative medicine. Transfer of skeletal myoblasts into decompensated hearts has been performed through intramyocardial injection. However, the achievement of both a cardiomyocyte differentiation and a precise integration of the injected cells into the myocardial wall, in order to augment synchronized contractility and avoid potentially life-threatening alterations in the electrical conduction of the heart, still remains a major target to be pursued. Recently, Granulocytes-Colony Stimulating Factor (G-CSF) fueled the interest of researchers for its direct effect on cardiomyocytes, inhibiting apoptosis and remodelling in the failing heart, and protecting from ventricular arrhythmias through the upregulation of Connexin 43 (Cx43). We propose a tissue engineering approach concerning the fabrication of an electrospun cardiac graft functionalized with G-CSF, in order to provide the correct signalling sequence to orientate myoblast differentiation and exert important systemic and local effects, positively modulating the infarction microenvironment.

Materials and Methods
An electrospinning equipment was used (DynaSpin, Biomatica, Italy) to produce PLLA/GCSF nanofibrous scaffolds. Microstructure of the obtained membranes was evaluated by SEM microscopy. Release of G-CSF from the scaffold was assessed by ELISA method. C2C12 mouse skeletal myoblast cell line (ATCC:CRL-1772) was seeded on PLLA/GCSF scaffolds for 48 hrs. Biological assessments including cell viability, proliferation and differentiation were performed. To assess differentiation, immunofluorescence, western blotting, flow cytometry for Connexin43 (Cx43) and Transmission Electron Microscopy were used. One-way ANOVA was performed to compare groups with different treatments, followed by multiple pairwise comparison procedure (Tukey test).

Results
Biological assays demonstrated the induction of Cx43 expression along with morphostructural changes resulting in cell elongation and appearance of cellular junctions resembling the usual cardiomyocyte arrangement at the ultrastructural level.

Discussion and Conclusions
The possibility of fabricating extracellular matrix-mimicking scaffolds able to promote myoblast precommitment toward myocardiac lineage and mitigate the hazardous environment of the damaged myocardium, represents an interesting strategy in cardiac tissue engineering.

Disclosures
Authors declare no conflict of interest