Aims of the study: We hypothesized that the optimal source of cell for vascular regeneration will be the progenitor cells derived from human embryonic stem cells (ESCs) which can differentiate both into endothelial cells (ECs) as well as vascular smooth muscle cells (SMCs). We propose to test if the population of human ESCs, H9 cell line, can serve this role.
Material and methods: Human ESCs were cocultured with stromal cells S17, M2-10B4 or Wnt1 expressing M2-10B4 cell line to generate a CD34+ cell population. After that, CD34+ cells were sorted and cultured in media containing specific cytokines to generate ECs. To induce SMC differentiation from ECs, culture conditions were changed to media containing platelet-derived growth factor-BB (PDGF-BB) and transforming growth factor-beta 1 (TGF-b1). Phenotypic and functional characteristics of these populations were demonstrated by flow cytometry, immunohistochemistry, QRT-PCR, tube formation assay, and response to calcium signaling agonists.
Results: CD34+ vascular progenitor cells derived from human ESCs give rise to ECs and SMCs. These two populations express cell specific transcripts and proteins, exhibit intracellular calcium in response to various agonists, and form robust tube-like structures when cocultured in Matrigel. Wnt1 overexpressing stromal cells produced an increased number of progenitor cells.
Conclusions: Here, we demostrate great potential of human ESCs (H9 cell line) to differentiate into both ECs and SMCs in a novel three-phase culture system. The ability to generate large numbers of ECs and SMCs from a single vascular progenitor cell population is promising for therapeutic use. The stepwise differentiation outlined in our work is an efficient, reproducible method with potential for large-scale cultures suitable for clinical applications.