N004
ISOLATION OF CARDIAC PRECURSOR CELLS FROM THE HUMAN FETAL HEART
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To identify a suitable source of human cardiac precursor cells (CPC) for replacement cell therapies, we established the conditions to isolate precursors from the human fetal heart. Ventricles were collected from donation following voluntary pregnancy termination at 12 weeks of gestation. Non-myocyte cells were isolated, and expanded in vitro. CPC were identified using immunostaining for Nkx2.5, Flk1 and Isl1, and by measuring the expression levels of these markers by real-time PCR. Proliferating cells demonstrated Nkx2.5 staining and no α-actinin expression. A significant fraction of Nkx2.5+ cells expressed Flk1. A few Nkx2.5+ cells were also Isl1+ whereas no Flk1+ cells expressed this marker. The capacity of expanded cells to produce differentiated cardiomyocytes, smooth muscle cells and endothelial cells was then tested by switching the culture to a differentiation serum-free medium. Differentiation into cardiomyocytes, smooth muscle and endothelial cells was evaluated by immunostaining for α-actinin, sm-MHC and CD31 respectively. In addition, expression of cardiac-specific (Nkx2.5, α-MHC, β-MHC), smooth muscle-specific (sm-MHC) and endothelial-specific (CD31) genes was measured using real-time PCR. Evidence for cardiogenic differentiation in the culture is supported by the significant increase in α-actinin+ cells (1% and 20% under expansion and differentiation conditions respectively) as well as by the upregulation of cardiac marker expression. Differentiation into smooth muscle cell type was also dramatically stimulated (0% and 4% respectively). Accordingly, sm-MHC expression was 25-fold increased. In contrast, no differentiation into endothelial cells was detected under these conditions. The engraftment and differentiation capacity of GFP-labeled CPC were tested in vivo by transfer into the heart of SCID mice after myocardial infarction. Seven days thereafter, engrafted cells were readily detected and demonstrated cardiac-commitment as shown by Nkx2.5 staining. At this stage, no differentiation into cardiomyocytes could be observed.

N005
POLYELECTROLYTE MULTILAYER FILMS: BOOSTER OF ENDOTHELIAL PROGENITOR CELL DIFFERENTIATION INTO ENDOTHELIAL CELLS
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With an autologous origin, high proliferation capacity and potentialities to differentiate into matures endothelial cells (EC), endothelial progenitor cells (EPC) have raised huge interest and offer new opportunities in vascular engineering. Currents protocols for isolation and differentiation of EPC requires at least 1 month to observe an endothelium-like morphology and about 2 months for confluent EC monolayer. We report here a new method to differentiate and expand EPC on polyelectrolyte multilayer films (PEM) which promotes rapid differentiation of EPC into EC.

Rabbit EPC isolated from leukocytes fraction were cultivated on a PEM or on Fibronectin (Fn) coated glass surfaces and jugular vein EC (JVEC) were used as control. Phase-contrast microscopy observations allowed to follow differentiation and morphological changes of the cells after 4, 14, 21 and 60 days. The evolution of the phenotype during the progressive differentiation of EPC into mature EC was also checked by confocal microscopy, verifying the expression of CD34, CD31, vWF and Dil-Ac-LDL expression.

After 4 days, the cells appeared to be round shaped without noticeable differences between the Fn and the PEM coated surface. Moreover, confocal microscopy observations have showed different stage of EPC going from early (CD34+/-) to late EPC (CD31+/- and vWF+/-) on both surfaces. After 14 days on PEM, EPC formed already a confluent monolayer and exhibited an endothelium-like morphology similar to those found in JVEC whereas on Fn, the similar cellular morphology was observed after 60 days of culture. Confocal microscopy observations after 14 days have indicated the full disappearance of CD34 expression, specific of haematopoietic phenotype whereas CD31, vWF and Dil-Ac-LDL, specific markers of matures EC, are expressed on both surfaces, signature of cellular differentiation. The grey levels quantification for these markers indicates an expression significatively higher only on PEM close to matures EC compare to Fn.

The present study describes an easy method for expansion and differentiation of EPC seeded on PEM and demonstrate that this strategy accelerate the differentiation into EC.