XV Congresso Nazionale della SOCIETA' ITALIANA DI RICERCHE CARDIOVASCOLARI (SIRC)
Imola (BO), 9 - 11 ottobre 2008 - Fondazione Cassa di Risparmio di Imola

Title: 3-D Fibrin Scaffold Improves Stemness of Human Peripheral Blood Endothelial Progenitor Cells

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Aims Fibrin is a natural biopolymer appealing for cell-based regenerative therapies, because it can support growth, migration and differentiation of different cell types. Endothelial progenitor cells (EPC) represent a very interesting alternative cell source for mature endothelial cells; the fact that can easily isolated from the peripheral blood, thereby eliminating donor morbidity, makes them ideal in applications in the field of regenerative medicine. We have demonstrated that fibrin can support EPC viability and growth.
Aim of this study was to evaluate if fibrin can affect EPC differentiation and stem cell markers expression.
Methods Fibrin was prepared mixing commercially available (Kedrion S.p.A. Lucca, Italy) fibrinogen (9 mg/ml) and thrombin (25 U/ml). Clot ultrastructure was investigated by scanning electron microscopy (SEM) and cryogenic SEM (CRYO-SEM) to measure fibre diameter and density. Clot elasticity was evaluated by atomic force microscopy (AFM), measuring the tip-sample force by cantilever displacement. EPC were obtained from peripheral blood and cultured on fibrin at the concentration of $1x10^6$cell/cm^2. Fibronectin coating was used as a control. Metabolic activity was assessed after 7 and 14 days by WST1 assay and viability by confocal microscopy (calcein incorporation). The expression of both endothelial (CD31, KDR, vWF, Ve-Cadherin) and stem cell markers (nanog, oct-4) was assessed by flow cytometry, confocal microscopy and Real Time RT-PCR.
Results SEM analysis revealed a nanometric fibrous structure, with mean fiber diameter of 165±4 nm and mean density of 95.9±0.2 %. CRYO-SEM suggested a reticulate structure with mesh-size up to 10 µm. Fibrin clot elasticity was 1.78 MPa, as in literature. WST1 assay showed that fibrin increased EPC metabolic activity as compared to fibronectin (fibrin: 0.606±0.056 a.u. vs. fibronectin: 0.311±0.067). Calcein staining demonstrated that EPC were still viable at 14 days. Flow cytometry showed the expression of endothelial markers (CD31=41.8±8.4%; vWF=32.3±3.0%; KDR=89.3±3.7%; VE-Cadherin=41.2±3.8%), confirmed also by confocal microscopy and Real Time RT-PCR. Interestingly, nanog and oct-4 (embryonic stem cell markers) expression was significantly greater on fibrin ($p<0.001$) as compared to fibronectin.
Conclusions These findings suggest that fibrin it is not only a suitable scaffold for EPC growth and viability but also induces EPC differentiation. The observation that Nanog, known as the most important marker of stemness, is maintained longer than on fibronectin, may offer a surplus value to stem cell-based therapies.