Harnessing Cell:Materials Interactions to Develop Innovative Strategy for the Recruitment of Progenitor Cells

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Statement of Purpose Introduction: Endothelial progenitor cells (EPCs) are bone marrow derived cells that have the ability to differentiate into vascular cell lines. Endogenous factors like cytokines and growth factors mediate recruitment of EPCs into the circulation. Circulating EPCs have a wide array of functions in tissue regeneration, tissue remodeling and cancer progression and are therefore increasingly being studied in various diseases ranging from ischemia, diabetic retinopathy, and cancer. The discovery that these cells can be mobilized from their bone marrow niche to sites of inflammation and tumor to induce neovasculogenesis has afforded a novel opportunity to develop cutting edge therapies. However, current have shown marginal success due to the selective use of individual agents and poor time residency at the site of injection. We discovered that a combination of polymeric materials and cells could be used as a platform technology to induce mobilization of EPC and recruit them to a site of interest in the body. By embedding cells within a 3D matrix we are able to tailor their release of multiple factors inducing recruitment of progenitor cells.

Methods: *Cell culture and morphologic analysis:* Human aortic endothelial cells (EC) were grown on gelatin-coated culture plates or in 3D gelatin matrices for 14 days. Cytoskeleton morphology was identified with immunofluorescent labeling (e.g. for actin and vinculin). *Cell secretion:* Conditioned media (CM) was gathered from cells in 2D or 3D cultures and secretome levels were measured by multiplex ELISA. *EPC recruitment test - In vitro:* Paracrine effect of secretome was tested by exposing cord blood (CB) or peripheral blood (PC)-EPCs seeded on the top membrane of a transwell plate to CM from 3D-EC and 2D-EC. After 24h incubation, the number of cells migrated through the membrane toward the CM was evaluated. *In vivo:* CM from 3D-EC and 2D-EC was injected in the peritoneal cavity of black mice, after 24hour peritoneal lavage was performed and extracted cells analyzed by three-markers FACS. EPC were determined as $CD45^{-}$, $CD34^{+}$ and $Flk\text{-}^{1+}$ cells. In a separate study, we induced hind limb ischemia in mice using femoral artery ligation. Conditioned media from 2D or 3D cultures was delivered to the ischemic limb using local delivery from alginate beads. The extent of recovery of perfusion and neovascularization was quantified using laser speckle imaging and histological analysis.

Results: We have previously shown that architecture of the underlying substratum determines the cellular morphology and imposes a structural alignment within EC for actin filaments and focal adhesion protein such as vinculin distinct from what is observed in 2D culture (1). In addition to morphological differences, secretome of ECs in 3D matrices differs from ECs in 2D. Release of active agents known to be involved in EPC recruitment, such as HGF and PDGF-BB, is up-regulated in 3D-ECs compared to 2D-ECs. Simultaneously, inflammatory-inducing factors are down-regulated with 2-fold lower release of TNF-a (Fig. 1A). In vitro migration assay clearly demonstrate the paracrine effect of the secreted factors resulting in a full migration of cells toward the bottom well when in presence of 3D-ECs CM (Fig. 1B).

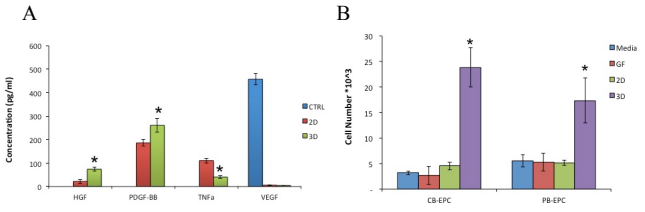

Figure 1. A) Released concentration of HGF and PDGF-BB growth factors increases in 3D-EC compared to 2D-ECm while TNF-a levels are reduced. B) Factors secreted from 3D-EC are able to paracrine recruiting all the EPCs regardless of their tissue of extraction.

Injection of CM from 3D-EC cultured for 14days resulted in a 9-fold increase in the in vivo mobilization of EPC to the site of injection in healthy mice when compared to 2D-EC (Fig.2A), and slightly improved blood flow in ischemic mice after 5 days of treatment (Fig.2B).

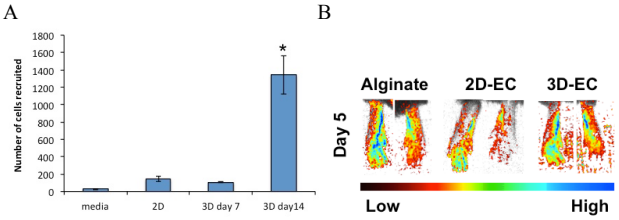

Figure 2. A) EPC recruited at the site of intraperitoneal injection for different conditions. B) Laser speckle imaging of perfusion in ischemic mice after 5 days of treatment with selected CM.

Additional screening of the miRnome is under evaluation to point out genetic differences between 2D- and 3D-EC responsible for the increased potential in recruiting progenitor cells.

Conclusions: Interactions of ECs with the surrounding substratum alter their capacity to exert paracrine control over recruitment of progenitor cells, like circulating EPC. Remodeling of the cytoskeleton, induced by substratum architecture, alters signaling pathways, which may account for subsequently tuning the release of growth factors. Taken together, our results demonstrate that EC-substrate interactions are potent regulators of endothelial function and can have a powerful effect on the new cutting-edge therapies to fight inflammatory diseases and promote tissue regenerative process.

References:

(1) Indolfi L., Biomaterials, 2012, 33(29):7019-27

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