Endothelial Cell -Substratum Interactions Control Monocyte Adhesion through a Src and MCP-1 Mediated Pathway

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Statement of Purpose: The endothelium is a powerful regulator of vascular homeostasis. Loss of endothelial cell (EC) quiescence facilitates pathophysiological processes ranging from atherosclerosis to inflammation. The conventional paradigm is that the confluent, intact endothelium ensures healthy vessels, while subconfluent ECs incite inflammation. However, this paradigm was established primarily from studies in two-dimensional (2D) culture conditions. *In-vivo*, EC reside in a complex three-dimensional (3D) environment whose micro-architecture changes with location in the vascular tree and function of surrounding tissues. In this scenario, tissue engineering constructs, as tools to investigating the local cell-substratum interactions, help to propel the understanding of this biologically relevant inter-relationship in a mechanistic way. We demonstrated that EC-substratum interactions in matrices with specific 3D microarchitecture can potentiate a dramatic transition of EC to a regulatory phenotype. This work examined the underlying mechanisms by which substratum architecture regulates EC interactions with monocytes and, ultimately, the inflammatory process that ensure.

Methods: *Cell culture and morphologic analysis:* Human aortic ECs 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) from 2D or 3D cultures was gathered from the cells and Monocyte Chemotactic Protein-1 (MCP-1) levels were measured by ELISA. *Monocyte adhesion test:* Calcein dye-labeled monocytic THP-1 cells were incubated with CM from 2D or 3D. These THP-1 cells were then applied to a confluent monolayer of TNF-α treated HUVECs, visualized by fluorescence microscopy and monocyte adhesion quantified by cell lysis and fluorimetric analysis.
Src inhibition and its role in monocyte adhesion: In parallel experiments, an inhibitor of Src pathway (PP2) was added to the 2D and 3D EC culture media for 24h, the media changed and CM taken after 24 hours. Similar experiments for cell morphology and THP-1 adhesion using this media were then performed.

Results: EC within matrices conform to the morphology of the underlying substratum by bending or wrapping around the struts of the gelatin network. The 3D substratum architecture imposes a structural alignment within EC for actin filaments and focal adhesion protein such as vinculin distinct from what is observed in 2D culture (Figure 1). In addition to morphological differences, ECs in 3D matrices secrete 8-fold less MCP-1 (289±61 pg/10^5cells) than EC in 2D culture (2526±740 pg/10^5cells, $p<0.05$ vs 3D). Monocyte adhesion to TNF-α activated HUVEC monolayers followed MCP-1 levels (R^2= 0.81). CM from EC within 3D matrices reduced monocytes adhesion of 4.7-fold more than CM from EC on 2D surfaces (70±5 vs 15±1 %, $p<0.05$, Figure 2).

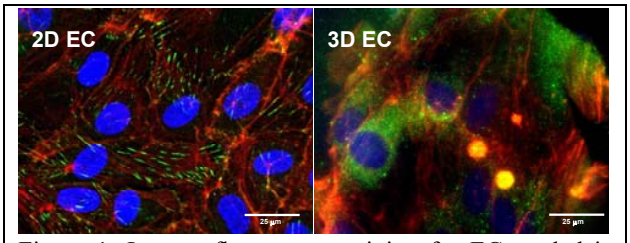

Figure 1: Immunofluorescent staining for EC seeded in 2D or 3D gelatin environment. Actin is labeled in red, vinculin in green and DAPI in blue.

THP-1 cell effects were mediated by MCP-1 through Src. Inhibition of the Src pathway enhanced monocyte adhesion reduction (now 42±1 % of control in 2D and 10±1% in 3D, $p<0.05$) (Fig 2) and decreased MCP-1 expression even further in both states; 348±100 vs 30±12 pg/10^5cells, 2 vs 3D ($p<0.05$). Secreted MCP-1 levels from PP2-treated EC in 2D approached to the basal levels of EC in 3D (348±50 vs 289±61 pg/10^5cells, $p=0.12$). Additionally, inhibition of Src pathway in 2D-seeded EC resulted in cytoskeletal architecture remodeling leading to a distribution of actin fibers and vinculin more similar to what present in 3D matrices.

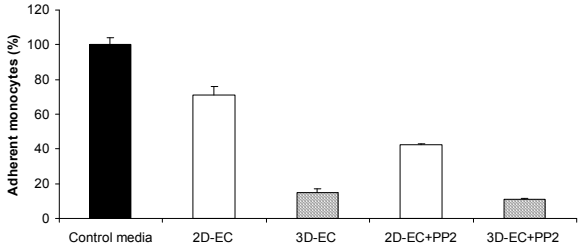

Figure 2. Src inhibition enhanced EC inhibition of THP-1 cells adhesion over a TNF-α activated HUVECs monolayer respect to control (42±1 % in 2D and 10±1% in 3D) compared to non treated EC (70±5 vs 15±1%)..

Conclusions: Interactions of ECs with the surrounding substratum alter their capacity to exert paracrine control over adjacent and contacting cells like monocytes. Remodeling of the cytoskeleton, induced by substratum architecture, alters signaling through the Src pathway which may account for subsequent reduction in monocyte adhesion. Taken together, our results demonstrate that EC-substrate interactions are potent regulators of endothelial function and can have a powerful effect on the inflammatory processes underlying diseases such as atherosclerosis and chronic inflammation.