Embryonic stem cells (ESC) have been explored as tools for studying development, as well as, potential sources for a large number of therapies in regenerative medicine. Traditionally, ESC are cultured on tissue culture plastic, however; it has been recently shown that the stiffness of the environmental substrate can direct the cells towards various cell lineages. Our laboratory is specifically interested in examining the combined roles of biochemical and physical signaling in cardiac and vascular cell fate and patterning these vascular cells into vascular branch-like tress. Using our novel mouse ESC that expresses a GFP reporter under the Tie-2 and an RFP reporter under alpha smooth muscle actin and serum-free induction mediums, we examined the role of stiffness in the diverging fate of Flk-1+ vascular progenitor cells. The results indicate that both of the Flk-1+ vascular progenitor cells and human umbilical vein endothelial cells (HUVEC) preferentially adhere to 10 kPa compared with the 1 kPa, and 34 kPa compared with the 10 kPa. We also observed both the GFP/Tie-2+ endothelial-like and RFP+ smooth muscle-like cells outgrowths from the Flk-1+ cells, with the GFP/Tie-2+ cells dominating the cultures, supporting the role of stiffness in vascular fate. Next, we generated a vascular fractal-like pattern reverse mold and have stamped fibronectin vascular pattern onto non-tissue culture treated plastic. Using these combined technologies, we have been able to generate vascular branching patterns with our ESC-derived EC.