The profound effects of ECM-associated biochemical signals on cell behavior have long been recognized. However, the impact of these biochemical signals on stem cell or progenitor cell differentiation have proven difficult to explore in a controlled manner, since most synthetic and natural materials adsorb a range of plasma proteins from the cell culture media. These adsorbed proteins are major determinants of cell behavior, in addition to any molecule deliberately added by the researcher. For controlled investigation of the effects of specific biochemical stimuli on cellular response, we therefore need a scaffold material that is essentially “non-biofouling”. Poly(ethylene glycol) diacrylate (PEGDA) is a ”non-biofouling” scaffold, and thus, in the absence of biochemical modification, presents a biochemical ”blank slate” to cells. The photoactivity of PEGDA permits desired biochemical moieties to be readily conjugated into the PEGDA hydrogels at defined levels. In the present work, we investigated the efficacy of defined biochemical stimuli on driving mouse embryonic progenitor (10T½) cells toward a mature smooth muscle cell (SMC) phenotype. To explore these effects, mouse embryonic progenitor cells were incorporated into PEGDA hydrogels into which defined levels of collagen type IV, fibronectin, laminin, fibrinogen, or heparin had been conjugated. Each of these molecules have been implicated in SMC differentiation or in maintenance of a mature SMC phenotype. 10T½ mouse embryonic progenitor cells encapsulated in hydrogels containing heparin and fibrinogen showed significantly higher levels of SMC markers SM-a-actin, calponin h1, and SM-g-actin than gels containing laminin or fibronectin. Similarly, collagen and elastin levels were higher in the fibrinogen and heparin hydrogels than in laminin and fibronectin gels. Focused microarray analyses indicate that variable modulation of the Wnt pathway may underlie the differing responses observed in the present study.