

560d Influence of Matrix Architecture on Esc Differentiation and Proliferation

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Embryonic stem cells (ESCs) present promising cell source for tissue regeneration due to their differentiation potential. Although the substratum that ESCs adhere and the growth factors contained in the conditioned medium play critical role in regulating ESC differentiation, it is not clear how the matrix architecture such as pore size and material stiffness affect ESC differentiation. In this study we questioned how a material presented in two different forms affect ESC proliferation and differentiation, using a murine ESC line (CCEs) which proliferates without differentiation on gelatin coated surfaces in presence of Leukemia inhibitory factor (LIF). First, we investigated ESC-differentiation in the absence of LIF on gelatin, chitosan and gelatin-chitosan membranes. ESCs lost their SSEA-1 and Oct-4 expression after withdraw of LIF on all the membranes, probably due to differentiation. However, the presence of chitosan did not allow ESCs to adhere to the substratum, similarly to other mature cell types. Compared with cells proliferating fast on gelatin alone, ESCs on chitosan contained membranes formed cell aggregates. When the same substratum was presented in three dimensional porous scaffolds with similar porosities, SSEA-1 expression varied and cell viability was higher on chitosan scaffolds than chitosan-gelatin scaffolds. Further, ESCs were cultured in EC medium containing defined growth factors or with additional 30ng/mL VEGF added. Positive Flk-1 expression and uptake of LDL exhibited markers featuring ECs in both cultures were observed after two weeks of incubation, analyzed by immunostaining and flow cytometry. However, CD31 expression was not observed. These results suggest that cytokines facilitating EC proliferation can also play a crucial role in directing ES cell differentiation into EC. 3D chitosan scaffolds were used as template to accommodate ES differentiation in the presence of VEGF for two weeks. Actin staining results showed that ES derived cells tended to aggregate under the guidance of pore structures.