Chronic kidney disease is a major global health problem causing significant morbidity and mortality. Therapeutic options for end-stage kidney disease, specifically dialysis and transplantation, improve survival but are limited by insufficient access and secondary complications. Research in regenerative medicine, with the aim of generating functional kidney tissue from human pluripotent stem cells, offers an innovative, patient-specific approach to treating this condition. Human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells can differentiate into organs from all three germ layers of the embryo and are ideal starting substrates for the derivation of kidney tissue. Importantly, the identification of culture conditions which promote the maintenance of a differentiated kidney epithelial cell phenotype and enable the three-dimensional arrangement of these differentiated cells into complex kidney tubular structures will be paramount to the successful generation of functional kidney tissue. The extracellular matrix (ECM) is an important component of the kidney architecture which may regulate its development, differentiation, and physiology. The primary aim of this work is to examine the effects of 2D and 3D ECM scaffolds on kidney differentiation from human ES and iPS cells.

In the first part of this aim, we will investigate the effects of 2D ECM on the differentiation of human ES and iPS cells into cells of the kidney lineage. Our laboratory has identified serum-free monolayer culture conditions on Matrigel® matrix (Corning) which efficiently induce differentiation of human ES/iPS cells into progenitor cells expressing PAX2 and LHX1, markers of intermediate mesoderm (IM), the embryonic tissue which gives rise to the kidneys. These cells, in the absence of additional external differentiation cues, give rise to polarized, ciliated tubular epithelial structures that express markers of kidney proximal tubular cells. However, these tubular structures dilate, become cystic, and lose their marker expression with prolonged culture, implying that specific culture conditions need to be defined to promote and maintain their differentiated phenotype. We are currently screening candidate growth factors and small molecules, including those available from BD such as human recombinant bFGF, EGF, IGF-I, and TGF-beta, to more efficiently promote tubular formation and kidney marker expression. While Matrigel is sufficient to allow the formation of tubular structures, we hypothesize that a combination of ECM proteins that are primary constituents of kidney basement membranes will promote formation and maturation of these tubular structures. To determine the optimal ECM substrate for this purpose, we will first test the effects of single ECM protein substrates using BioCoat® Collagen I, Collagen IV, Fibronectin, and Laminin multiwell plates (Corning). The pre-coated 96-well microplates will make it faster, simpler, and more reproducible to vary the ECM substrate while simultaneously screening multiple growth factor conditions. We will also screen combinations of the PureCoat™ ECM proteins Types I and IV Collagen, Fibronectin, and Laminin (Corning) at varying concentrations on 96-well microplates. To test conditions consistent with good manufacturing practices for potential medical application, differentiation will also be tested on Vitronectin (Corning), a xeno-free substrate for pluripotent stem cells. Human
ES/iPS cells will be seeded and differentiated on these strata using our established protocol and assayed at three different time points. Our readouts will be 1) tubular formation, as assayed by quantitation of tubular structures per microwell, and 2) immunocytochemistry for markers of nephron segment-specific epithelial cells, including kidney-specific protein (KSP), Lotus tetragonolobus lectin (LTL), and aquaporin-1 for proximal tubular cells; Tamm-Horsfall protein for Loop of Henle and distal tubular cells; and Dolichos biflorus agglutinin (DBA) for collecting duct cells.

We have observed that sandwiching differentiating IM cells in a 3D gel of Matrigel increases the number of tubular structures formed, suggesting that 3D culture may enhance differentiation. In the second part of this aim, we will identify a 3D ECM microenvironment which will promote the formation of kidney tubules from differentiated human ES/iPS cells. We will take full advantage of the convenience and flexibility of the PuraMatrix™ peptide hydrogel system (Corning), which will enable us to perform 3D cell encapsulation with ECM protein supplementation. The ability of PuraMatrix supplemented with varying concentrations of BioCoat Types I and IV Collagen, Fibronectin, Vitronectin, and/or Laminin to induce tubule formation will be tested using an established human kidney proximal tubular cell line (HKC-8), which has been shown to form tube-like structures in 3D Matrigel. Tubular structures will be quantified and immunostained for KSP and LTL to confirm their identity as proximal tubular cells. 3D PuraMatrix preparations that significantly increase tubule formation in HKC-8 cells compared to the Matrigel-positive control will then be tested on human ES/iPS cells differentiated into PAX2+LHX1+IM cells using our protocol. The identification of a 3D microenvironment to promote kidney differentiation and tubular formation will be a novel and critical advance towards bioengineering functional human kidney tissue. Optimized 2D or 3D culture conditions may also reveal cystic growth phenotypes in iPS cells derived from patients with polycystic kidney disease, a common cause of kidney failure, when compared to iPS cells from healthy patients. BD Biosciences reagents have been integral to the success of our stem cell work, and the reagents awarded with this Stem Cell Grant will greatly facilitate our ability to execute the proposed studies.

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