

Abstract:

Traditional primary culture of renal cells involves enzymatic dissociation of kidney tissue followed by propagation of the cells on tissue-culture treated plastic with or without extracellular matrix coatings. In the present study, we examined the effects of three-dimensional (3D) architecture and perfusion on metabolism, phenotype, and tubular function of primary kidney cells in a variety of culture configurations. 3D architecture promoted cell-cell interaction and organization, as determined by scanning electron microscopy, histology, and confocal immunocytochemistry. The addition of perfusion to the culture system resulted in enhanced metabolic activity and a significant and sustained upregulation of genes associated with tubular function. Importantly, the tubular function of renal cells was confirmed in culture systems via the demonstration of megalin/cubilin-mediated uptake of albumin. In summary, dynamic 3D culture systems provide a means to examine tubular cell phenotype and function in an environment that better recapitulates *in vivo* biology.

Introduction:

Over 20 million people are currently diagnosed with Chronic Kidney Disease (CKD) in the US¹. A great deal of cost and morbidity are associated with the final stages of CKD. End-stage renal disease (ESRD) patients currently have the option of an organ transplant and/or dialysis to survive. Preventing progression to ESRD through a regenerative medicine alternative may help prevent adverse outcomes, improve quality of life, and reduce the cost of healthcare. Despite intense investigation into renal development and disease, the field lacks simple and accurate *in vitro* models of kidney biology. The availability of fresh whole kidney tissue provides an opportunity to study function at the cellular and molecular level in primary cells that have not been immortalized or cultured extensively. The perfused 3D kidney culture system presented herein allows the capture of architectural, histological, phenotypic, genotypic and functional changes in a controlled manner.

Results:

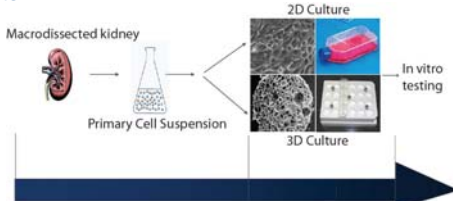


Figure 1. Isolation, culture, and expansion of primary kidney cells. A prototype Multiwell Perfusion System (MPS) of Becton, Dickinson and Company aids 3D culture with continuous media circulation.

References:

1. The United States Renal Data System. The United States Renal Data System Annual Data Report 2007. Available at: www.usrds.org/adr.htm. Accessed August 27, 2008.

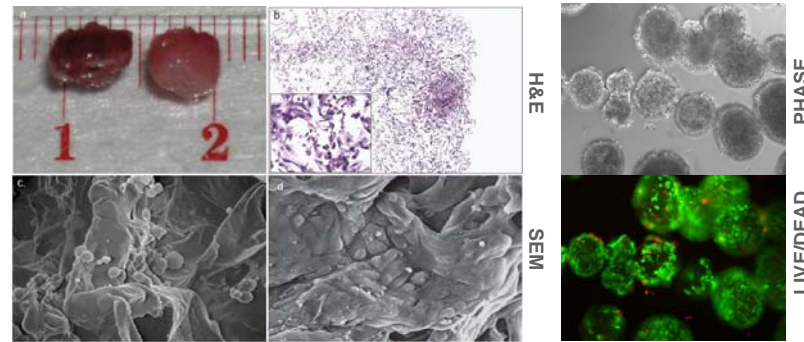


Figure 2. a. 5mm x 5mm porous scaffold post-culture perfused (left) and static (right), live cells stained with neutral red. b. H&E of perfused porous scaffold. c. and d. SEM of porous scaffold after 7 days of culture under static and perfused conditions respectively. Note the greater cellularity and organization of the perfused vs. static culture. Right panel, a representative live (green) dead (red) assay on primary kidney cells cultured on three-dimensional porous beads in a dynamic environment.

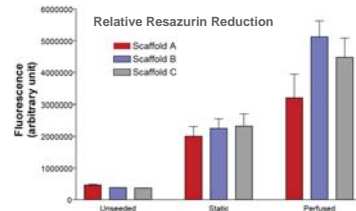


Figure 3. A Resazurin assay was used to assess metabolic activity in the scaffolds. Three seeded scaffold configurations (n=4) were cultured for (5) days in either perfused or static conditions. Enhanced resazurin activity was observed in all scaffold types examined.

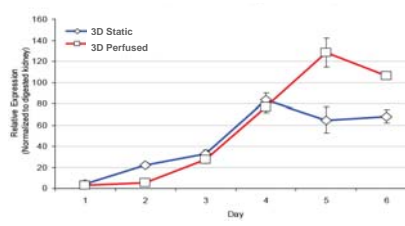


Figure 4. Gene expression of the tubular marker E-cadherin was measured from primary kidney cells cultured in 3D under static versus dynamic conditions over time. On days 5 and 6, tubular gene expression is significantly enhanced in dynamic culture.

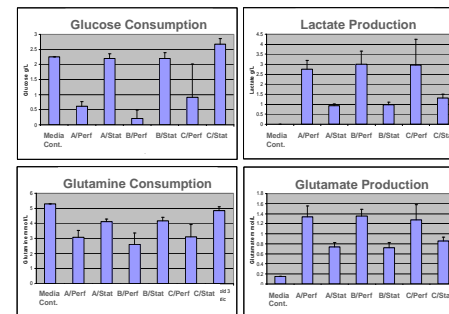


Figure 5. Conditioned media (n=4) was collected to examine consumption of glucose and glutamine by perfused and static 3D cultures of primary kidney cells on a Nova Bioprofile 400. Glutamine consumption and glutamate production were also enhanced with perfusion in all 3D configurations.

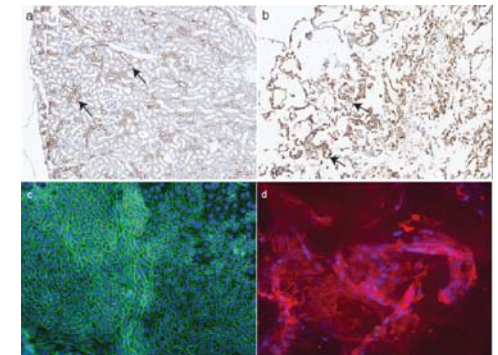


Figure 6. E-cadherin staining in a. normal rat tissue, b. on perfused Scaffold (B), c. 2D culture (E-cadherin, green), d. Confocal image of a perfused Scaffold B (E-cadherin, red).

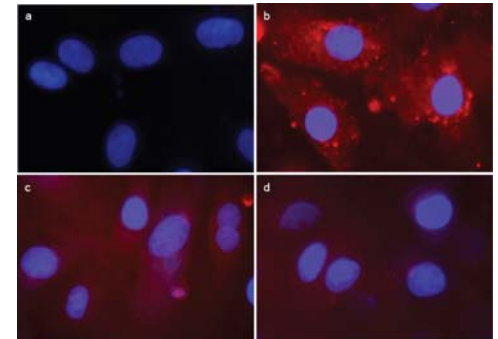


Figure 7. *Ex-vivo* tubular function via albumin uptake. Rat primary kidney cells (P1) in vitro. Nuclei stained with Hoechst (blue). a. Untreated. b. Human serum albumin-rhodamine (red) pulsed for 15 min at 10 ug/ml. c. Albumin pulsed at 4°C. d. Pretreatment with the Cubilin/Megalyn inhibitor, RAP.

Conclusions:

Three-dimensional, dynamic culture of renal cells *in vitro*:

- Promotes cellular interactions and may lead to the development of multi-cellular neo-tissues
- Provides a contextual model with which to study early tubular regeneration events at the cellular level