Hypoxic Exposure of Cultured Human Renal Cells

Induces mediators of cell migration and attachment and facilitates the repair of tubular cell monolayers in vitro

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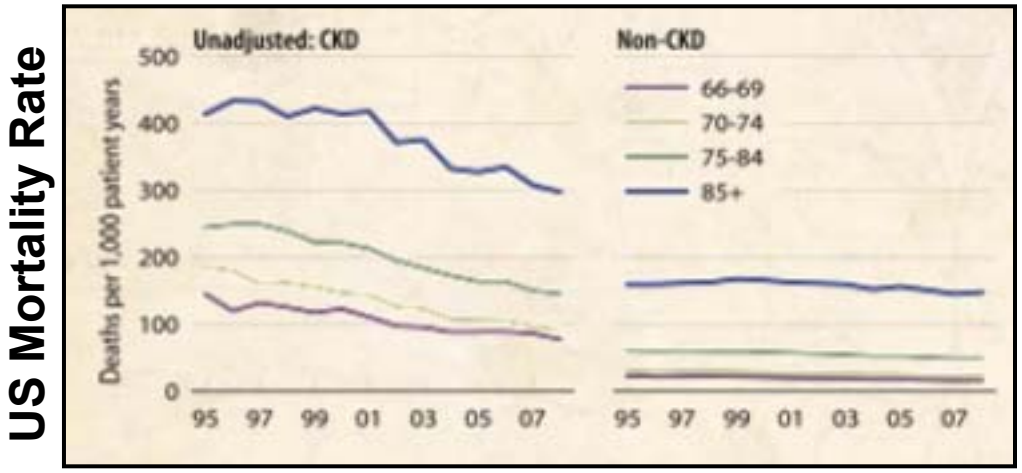
Experimental Biology, Washington, DC

Chronic Kidney Disease (CKD)
is a leading cause of morbidity and mortality



- ***11.5% of adults >20 years old (23M) have physiologic evidence of Chronic Kidney Disease (CKD)***

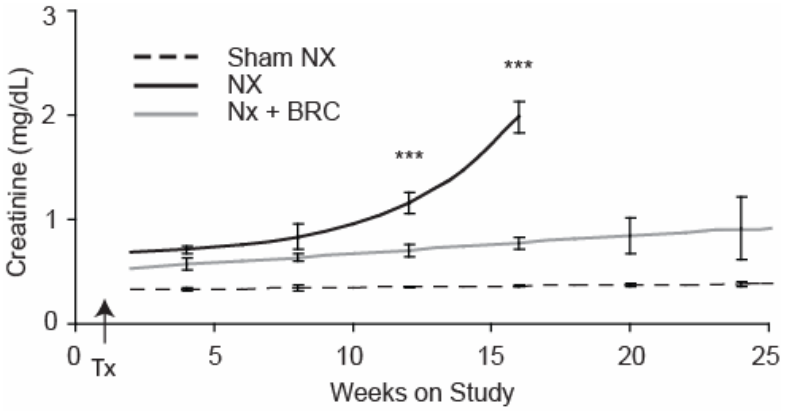
- ***In 2007***
  - *368,544 US residents were on dialysis*
  - *17,513 kidney transplants were performed (with >80,000 people on waiting list)*
  - *87,812 deaths occurred from End-Stage Renal Disease (ESRD)*

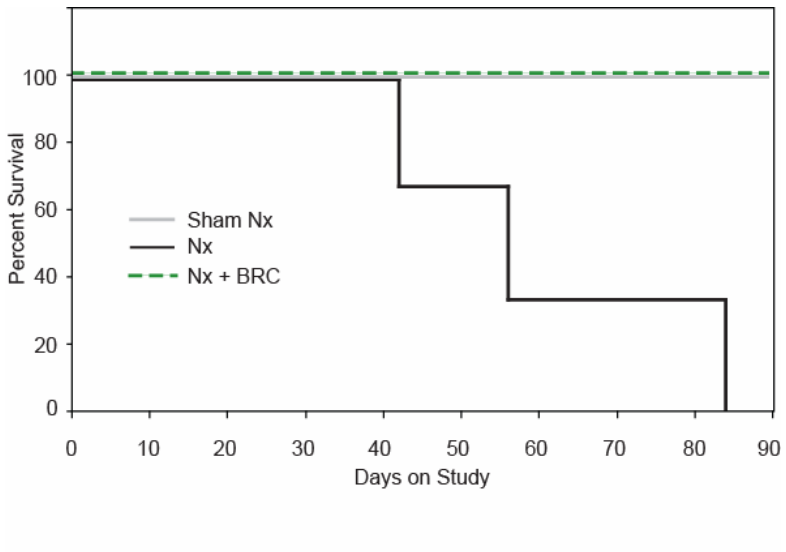
- ***New treatment options are needed***

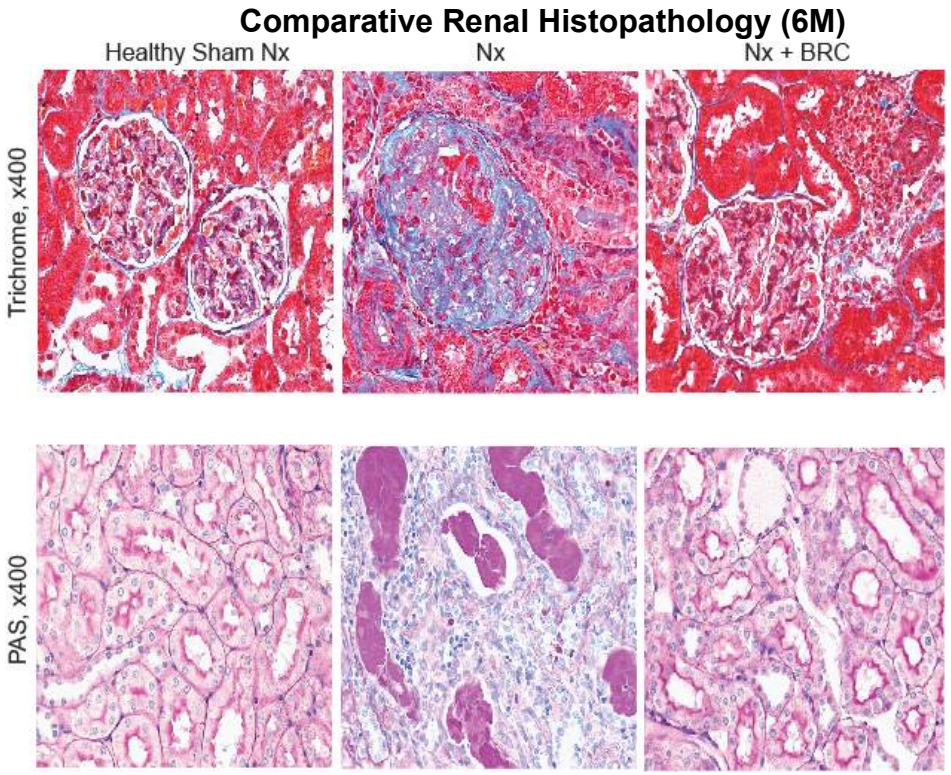
http://kidney.niddk.nih.gov/kudiseases/pubs/pdf/KUStatistics.pdf

Intra-renal Transplantation of Selected Bioactive Renal Cells

Enhanced survival and stabilized renal functions in CKD





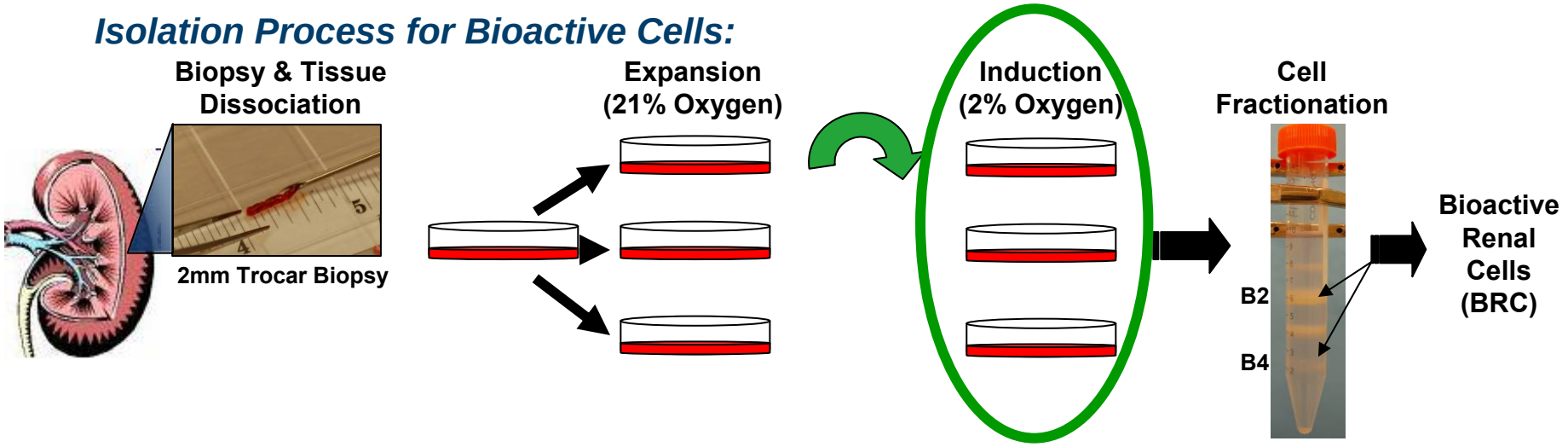


Selected renal cells are biologically active:

- *Stabilized glomerular filtration (GFR)*
- *Improved tubular function*
- *Prolonged survival*
- *Modulated fibrotic pathways*

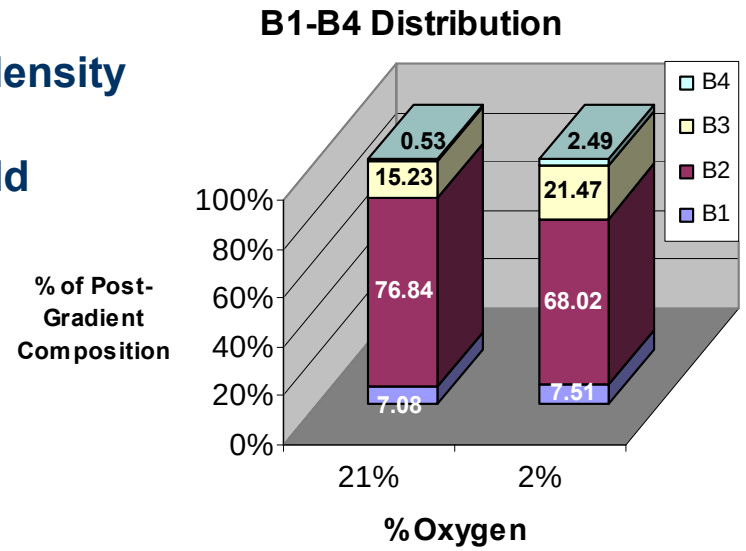
Exposure to Low Oxygen During Processing
Alters composition and function of selected cells

Isolation Process for Bioactive Cells:



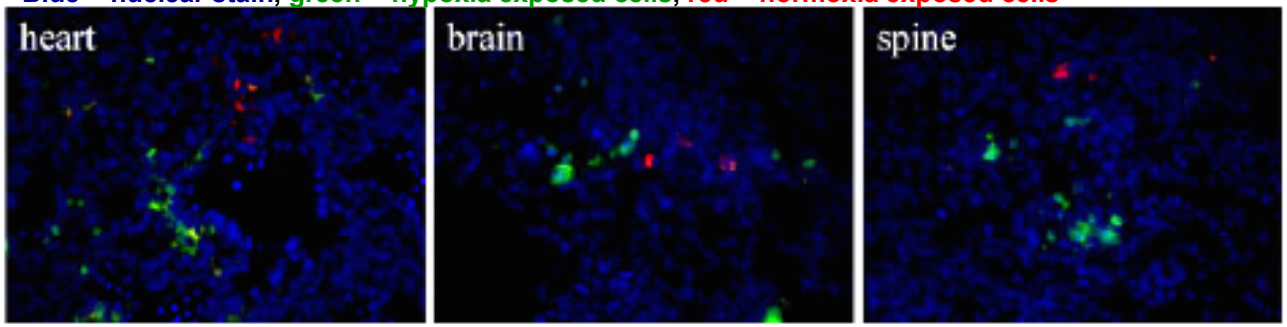
Exposure to 2% Oxygen:

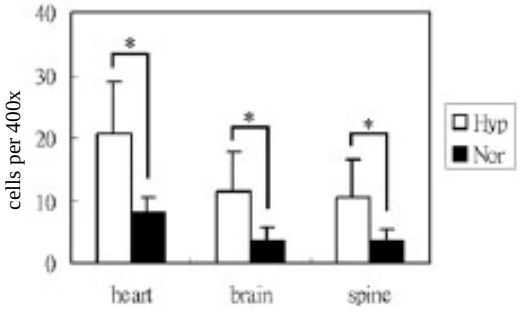
- Alters distribution of cells across density gradient
- Improves overall post-gradient yield
- Modulates oxygen-regulated gene expression*
  - Erythropoietin ↑
  - VEGF ↑
  - HIF1-alpha ↑
  - KDR(VEGFR2) ↑



Low-oxygen Induction of MSCs Prior to Implant
*Enhanced engraftment and function**

Blue = nuclear stain, **green = hypoxia exposed cells**, **red = normoxia exposed cells**





From: Hung et al., PLoS One 2:e416, 2007)

- *Increased expression of chemokine receptors and engraftment of MSCs in-vivo (*Hung et al. 2007 PLoS One 2:e416)*
- *Enhanced vascular regenerative response of MSCs in a hindlimb ischemia model (*Leroux et al. 2010 Mol Ther 18:1545)*
- *Induced cytokines and increased angiogenesis and heart function in MSC-seeded myocardial patch (*Huang et al. 2010 J Mol Cell Cardiol 48:702)*
- *Improved engraftment of MSCs after low-oxygen preconditioning in a damaged heart (*Noort et al. 2010 Panminerva Med 52:27)*

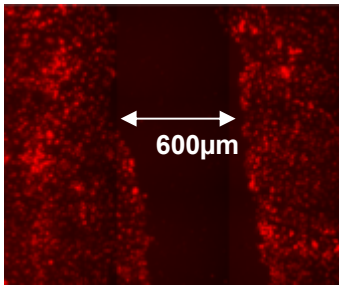
***Hypothesis*: In-process exposure to low oxygen enhances the ability of selected Bioactive Renal Cells to repair / regenerate damaged renal tubules.**

In vitro Evaluation of Renal Regenerative Response

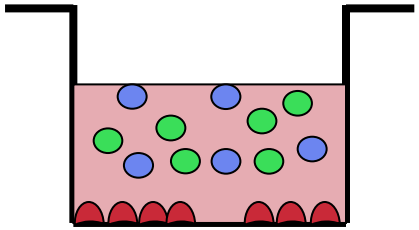
Development of assays for attachment & migration

Assay 1: Attachment

1. Label cells with fluorescent dyes
   - $2\%O_2$
   - $21\%O_2$
   - HK2 Tubular Monolayer (wounded)
2. Wound tubular cell monolayer



3. Add oxygen-exposed labeled cells

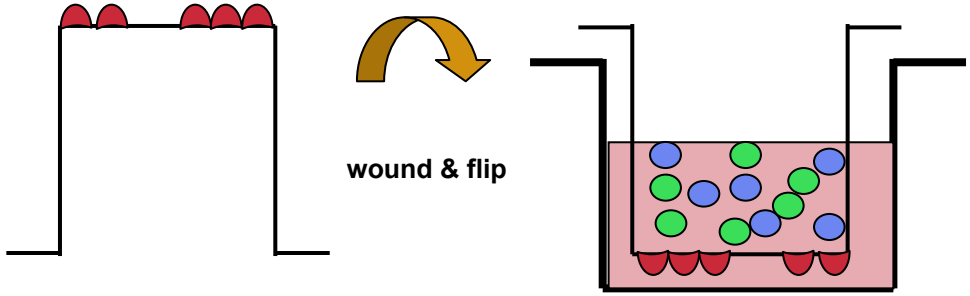


- 2%- and 21%- exposed BRC
- seeded equally @20K/cm^2
- Serum-Free media / 5%O_2 / 24hrs

4. Quantify cells that repair wound

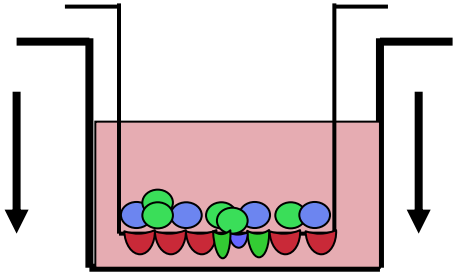
Assay 2: Migration and Repair of Tubular Cell Monolayer

1. Label cells with fluorescent dyes
2. Establish tubular cell monolayer on bottom of 8µm pore size transwell insert and wound
3. Add 2% and 21% oxygen exposed labeled cells



- Seeded equally @50K/cm^2
- Serum-free media / 5% O2 / 24 hrs

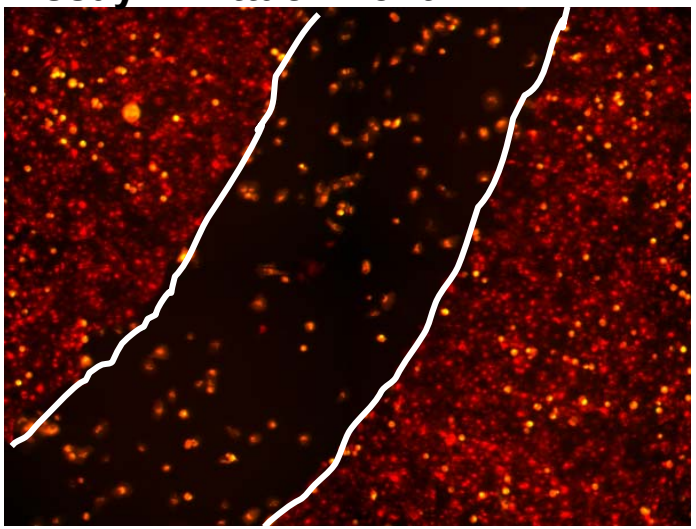
4. Quantify cells that migrate and repair wound



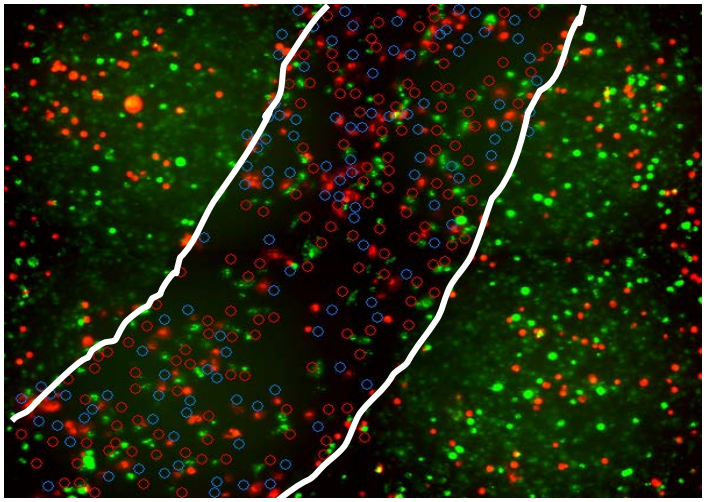
Repair of Damaged Tubular Epithelial Monolayers
Is enhanced in 2% oxygen-induced selected renal cells

Assay 1: Attachment

t-0hr

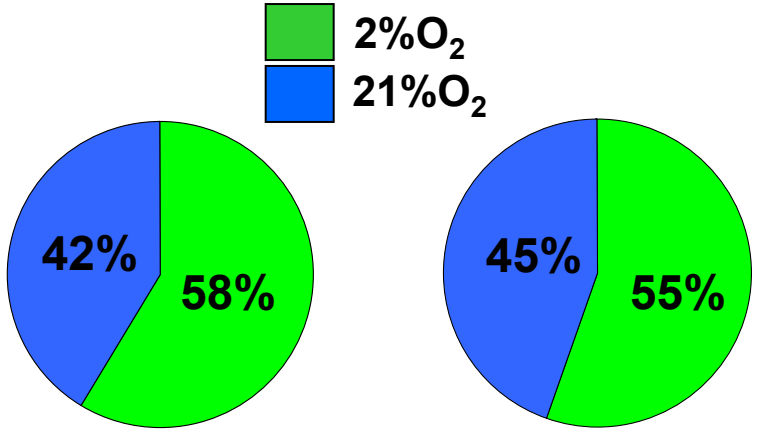


t-2hr



red circles = cells cultured 2%O2, blue circles= 21%O2

- **Quantitative Image Analysis (BD Pathway 855 BioImager)**
- **2% oxygen-induced BRCs**
  - attached more rapidly (2 hrs)
  - sustained a mild advantage for 24 hrs

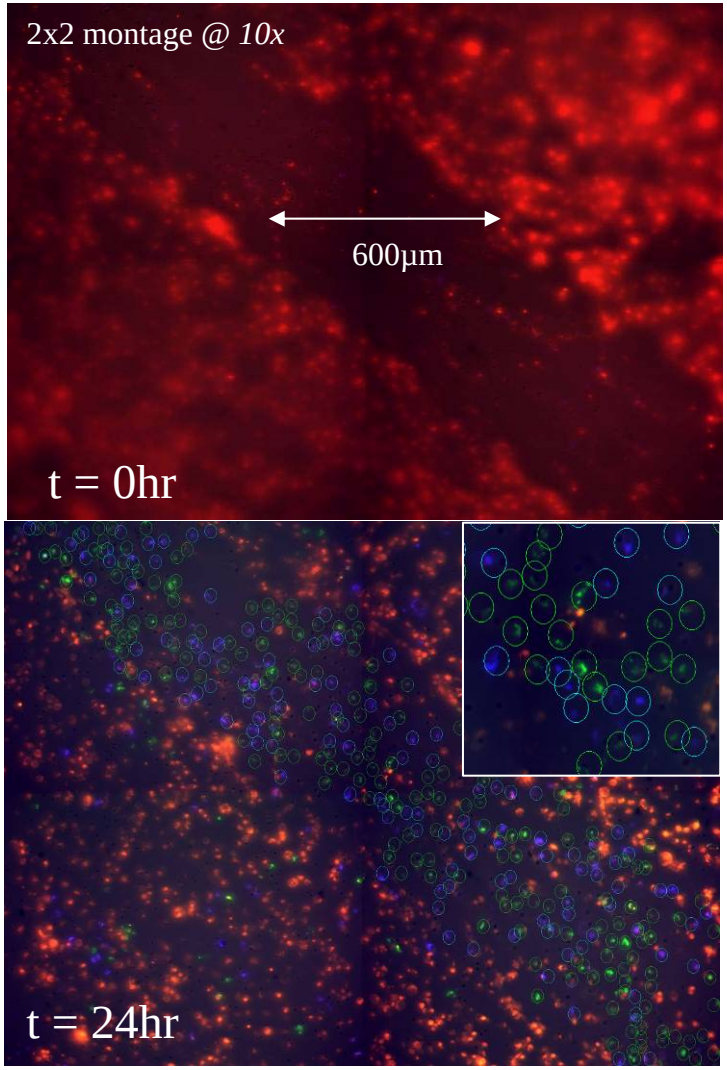


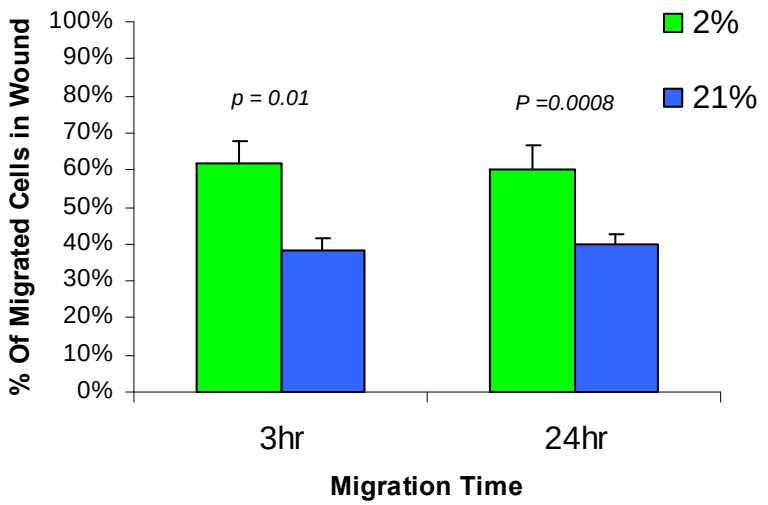
t-2hrs **t-24hrs**

Induction of Selected Renal Cells with 2% Oxygen

Enhanced migration response to damaged epithelium

Assay 2: Migration and Repair of Monolayer

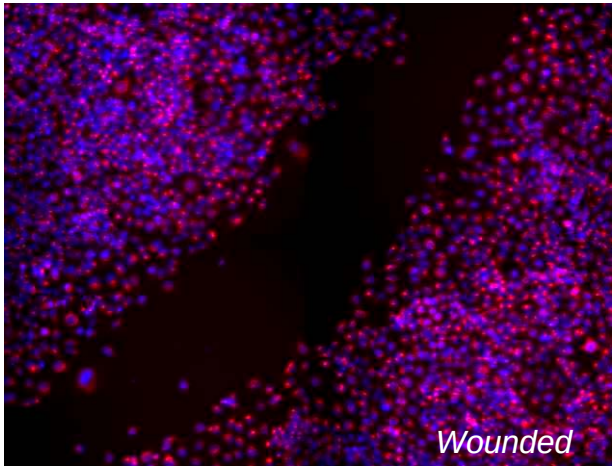


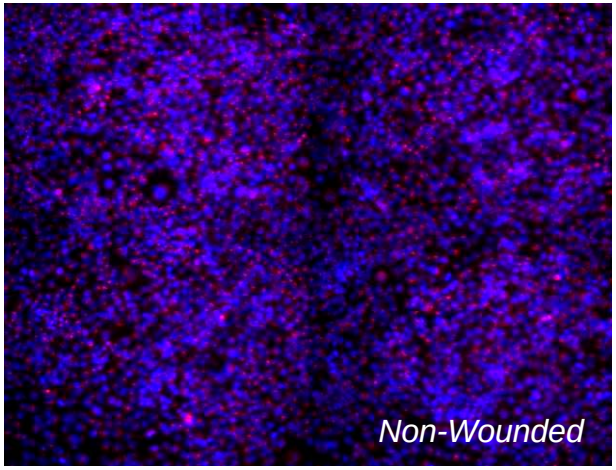


N=3	3hr		24hr	
	Average # cells	Average %	Average # cells	Average %
$2\%O_2$	26.33	61.51%	117.67	60.35%
$21\%O_2$	16.67	38.49%	76.33	39.65%

Quantitative image analysis using *Simple PCI*

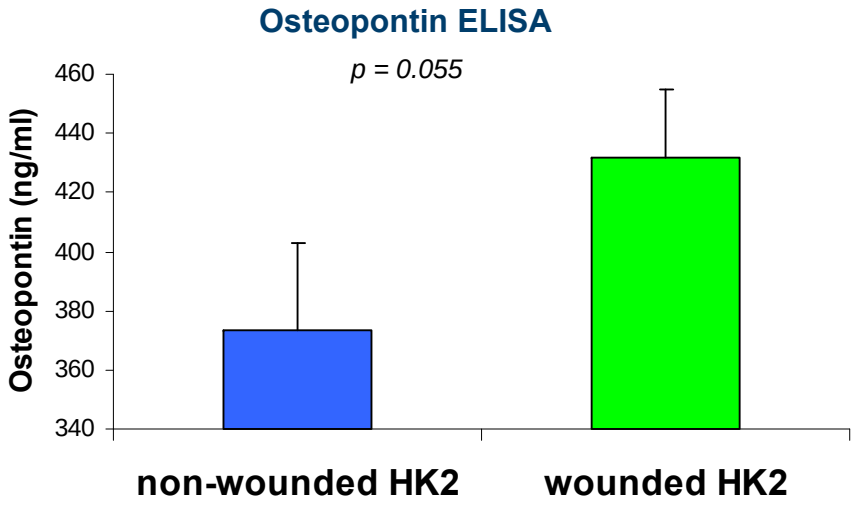
Osteopontin is Secreted by Tubular Cells *and upregulated in response to injury*



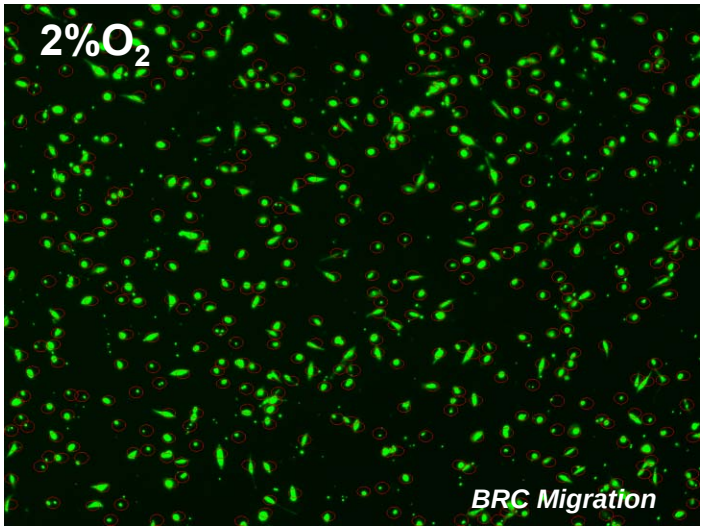


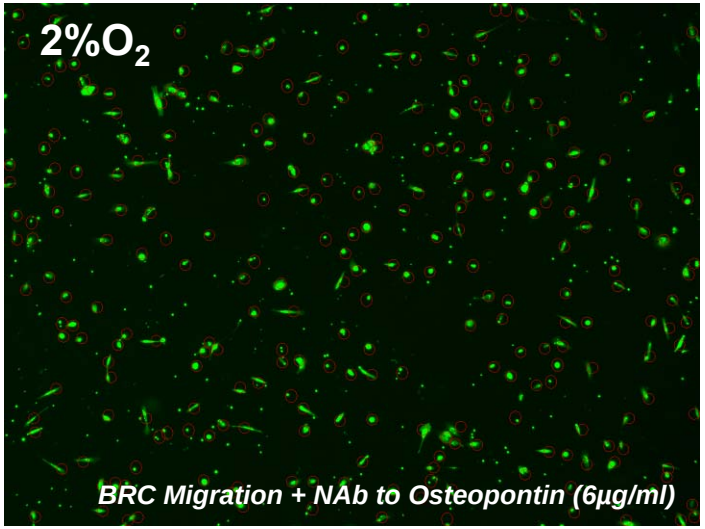
Osteopontin Immunocytochemistry: Hoechst nuclear stain (blue), Osteopontin (Red), 10x

- *Osteopontin is a secreted phosphorylated glycoprotein**
  - *Expressed in kidney tubules*
  - *Involved in adhesion and migration*
- *Osteopontin is upregulated by injury in established tubular cell monolayers*
  - *Immunoflluorescence*
  - *ELISA*



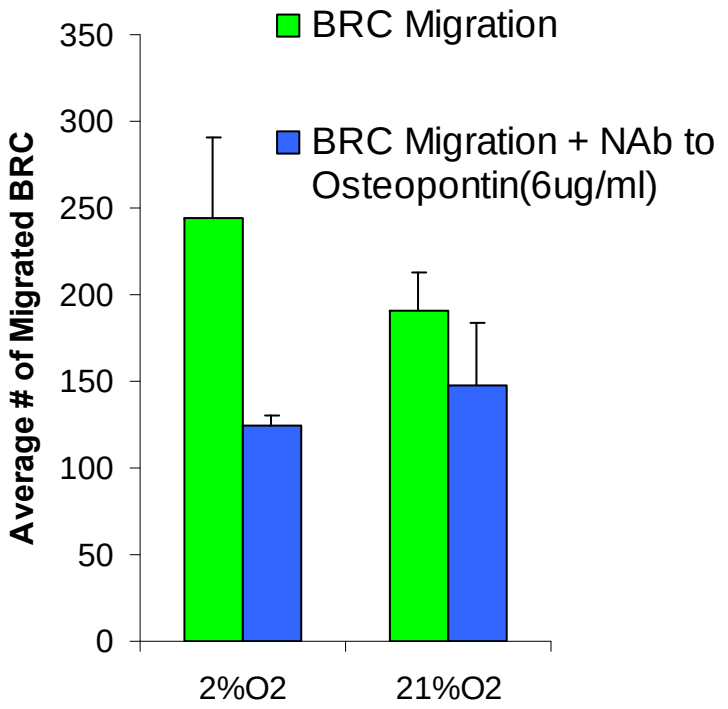
The Migratory Response of Selected Renal Cells
Is mediated in part by Osteopontin



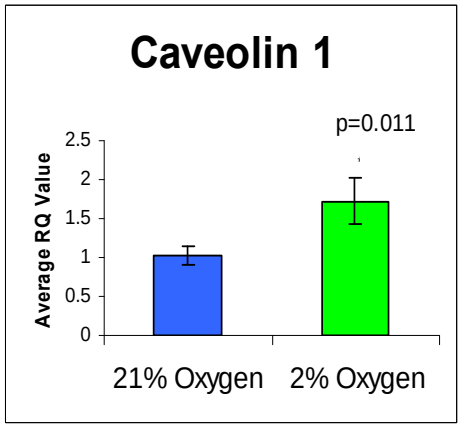


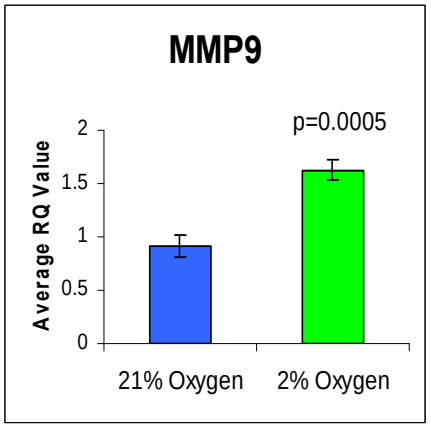
Green (Calcein AM) = migrated BRCs (5x)

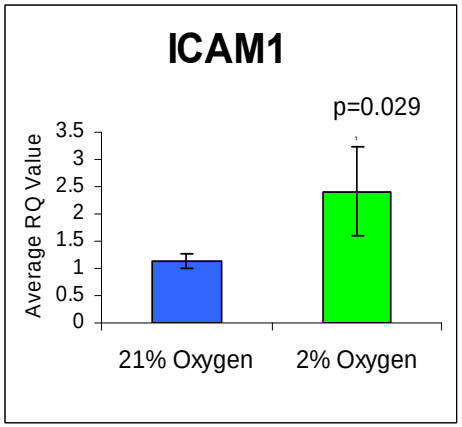
Neutralizing Antibodies (NAb) to Osteopontin reduce renal cell migration response by 50%

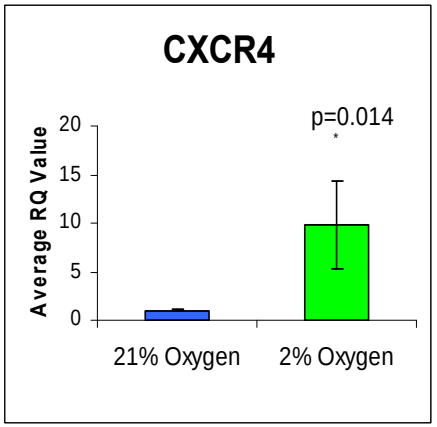


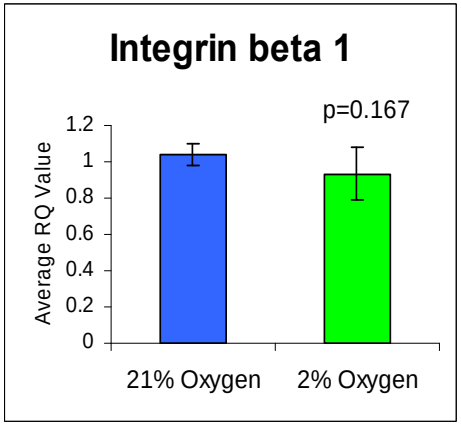
Low-oxygen Induction of Selected Bioactive Renal Cells
Modulates expression of tissue remodeling genes

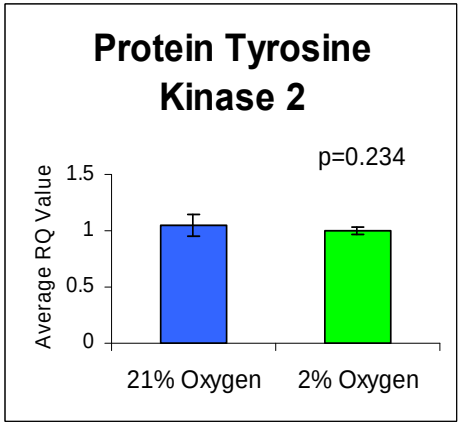


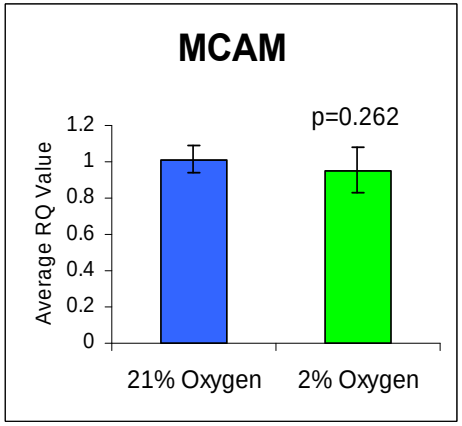








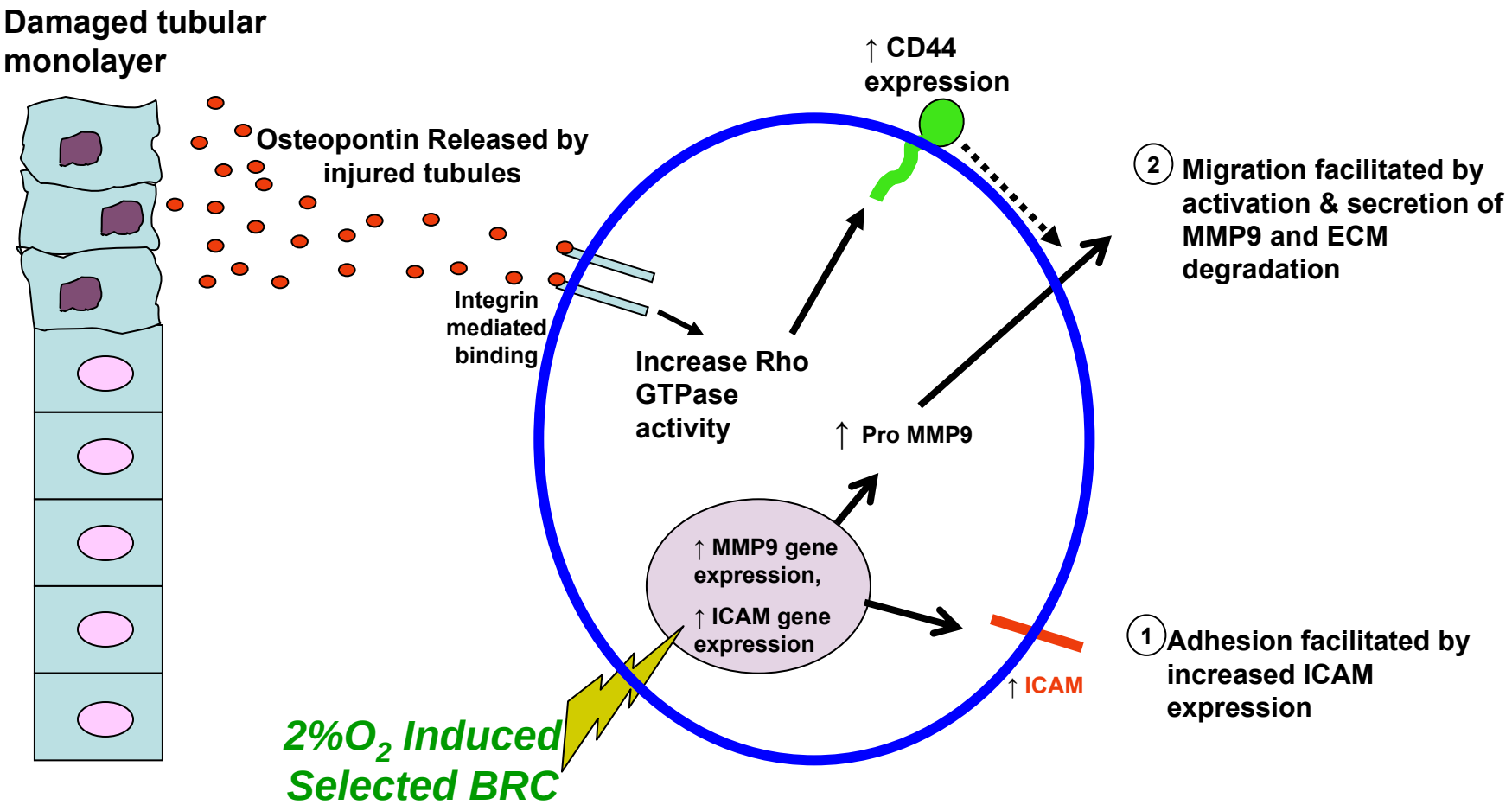




- ***Caveolin 1: scaffolding protein involved in modulation of integrin signaling***
- ***MMP9: metalloproteinase that facilitates migration through extracellular matrix degradation***
- ***ICAM1: Intercellular adhesion molecule associated with epithelial cell motility***
- ***CXCR4: chemokine surface receptor that mediates cell migration***

Low Oxygen Augments Bioactivity of Selected Renal Cells

Putative mechanism(s) in renal regeneration



Conclusions

- ***Selected Bioactive Renal Cells stabilized renal function and enhanced survival in a rodent model of progressive CKD***

- ***Low oxygen levels (2% O_2)***
  - *Enhanced post-culture recovery of selected regenerative cells*
  - *Enhanced cellular attachment and monolayer repair in response to tubular injury*
  - *Stimulated cellular migration in response to tubular injury*

- ***Cellular migration and attachment were mediated in part by osteopontin* in vitro**

- ***Low-oxygen upregulated integrins, secreted proteins, and cell adhesion molecules which mediate tissue remodeling, migration, and cell-cell communication***

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