Abstract: TH-PO761

Substrate Elasticity Governs Differentiation of Renal Tubule Cells in Prolonged Culture

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Background

Primary tubule epithelial cells rapidly dedifferentiate in culture and form a flattened epithelium lacking the brush border essential to apicobasal transport. This dedifferentiation undermines the use of cultured cells for drug screening and cell-based therapies. We hypothesized that substrate mechanical properties have a strong influence on differentiation in primary renal cell culture.

Methods

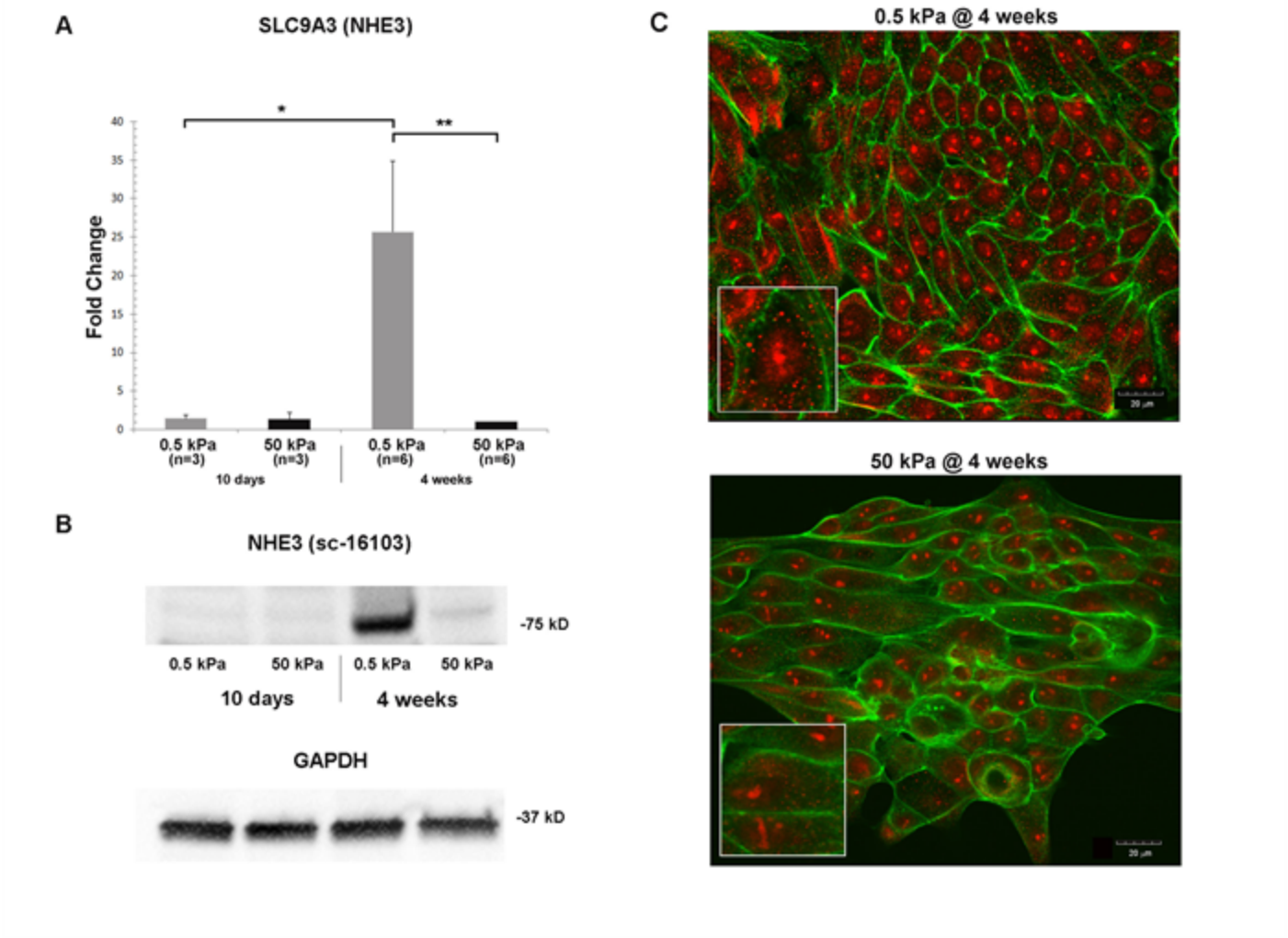
We cultured primary renal tubule cells on polyacrylamide hydrogels of varying elasticity for 2 and 4 weeks. We measured expression of key transporter proteins essential to renal tubule cell function at the transcript and protein level.

Results

Primary tubule cells cultured on soft substrates for two weeks did not show marked differences in transcript or protein levels for NHE3 or AQP1, but after 4 weeks in culture, NHE3 and AQP-1 were increased on 0.5kPa gels compared with 50 kPa gels. Smad2 phosphorylation increased with increasing substrate stiffness, suggesting a role for TGF-β signaling. Indeed, addition of TGF-β to cell culture media abolished the elasticity-dependent increase in NHE3.

Conclusion

These data support the hypothesis that scaffold elasticity is a critical factor in differentiation of renal tubule cells in culture.



NHE3 figure. *(A): Expression levels of SLC9A3 (NHE3) were determined by qPCR in primary HREC cultured on soft (0.5 kPa), and stiff (50 kPa) hydrogels. Cells were harvested at short (10 days) and long (28 days) time points. (B): NHE3 protein levels were determined by Western Blot analysis. (C): NHE3 (Red) and F-actin (Green) staining of HREC cells cultured on soft (0.5 kPa) and stiff (50 kPa) hydrogels for 4 weeks. * p=0.003, ** p=0.00002.*

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