Design of Integrin Specific Fibronectin-Type III Domains to Direct Epithelial Cell Phenotype
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Introduction
The extracellular matrix (ECM) provides important cues for directing cell fate, such as cell spreading, proliferation, and differentiation. Cells interact with their underlying ECM through transmembrane cell-surface receptors known as integrins, which bind to specific sequences on their ligand. One such binding sequence, Arg-Gly-Asp (RGD), is found in fibronectin (Fn) on the 10th type III repeat, and is known to interact with many integrins including αVβ3. Another critical integrin binding site, PHSRN, is found in Fn on the adjacent 9th type III repeat in close proximity to the RGD site and has been shown, in concert with the RGD site, to be critical for integrin α5β1 binding. We hypothesize that the Fn PHSRN site is critical to the regulation of epithelial cell phenotype, specifically the process of epithelial to mesenchymal transition (EMT), by directing integrin specificity. EMT is a critical cellular transition necessary for embryonic development of complex tissues and wound healing. We have previously created recombinant fragments of Fn that display “stabilized” PHSRN and RGD (Fn9*10), or RGD alone (Fn10) and demonstrated the power of these motifs for enhancing stem cell differentiation in 2D and 3D systems [1]. Here we show the ability of these Fn fragments to induce differences in epithelial cell phenotypes relevant to the formation/regeneration of complex tissues.

Materials and Methods
To analyze epithelial cell responses to the Fn fragments, alveolar epithelial precursor cells were cultured on the fragments and cell phenotype analyzed by cytoskeleton changes, epithelial cell contacts, changes in gene expression of epithelial and mesenchymal markers, and changes in cell circularity. Wound healing responses on Fn fragments were also analyzed.

Results
Cells adhered to Fn10 predominantly via αv integrins and showed a downregulation of epithelial markers and an upregulation of mesenchymal markers compared to cells cultured on Fn9*10 which engage mainly α3 and α5 integrins, indicating an integrin-specific shift toward a mesenchymal phenotype on Fn10 compared to Fn9*10. In addition, cells cultured on Fn10 lost cell-cell contacts as determined through E-cadherin staining and showed a decrease in circularity over the course of two days. A wound-healing assay indicated that cells cultured on Fn9*10 were significantly more efficient at closing the wound gap compared to cells cultured on Fn10 (p<0.001).

![Fig. 1. Relative expression of epithelial and mesenchymal genes on Fn fragments.](image)

Discussion and Conclusions
These studies demonstrate our ability to engineer Fn fragments that can induce integrin-specific responses leading to differences in epithelial phenotype. Upon further study, these Fn fragments, and others, could potentially be used to drive specific epithelial phenotypes for regenerative medicine technologies.

References

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Disclosures
Authors have nothing to disclose.