

### **A role for collagen I in regulating connexin-43 mediated hemichannel activity in the proximal region of the diabetic kidney**

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**Background and aims:** Tubulointerstitial fibrosis represents the key underlying pathology in diabetic kidney disease and is characterised by tubular injury and extracellular matrix remodelling. In diabetic nephropathy increased collagen I deposition is linked to inflammation and fibrosis, yet, we know little as to how collagen facilitates these effects. Pivotal to early tubular injury is impaired epithelial (E)-cadherin mediated cell adhesion; loss of which inhibits docking of connexin-mediated hemichannels and prevents gap junction intercellular communication. Uncoupled hemi-channels respond via increased release of adenosine triphosphate (ATP), an effect we have previously confirmed is linked to increased expression of inflammatory and fibrotic markers. The current study investigates if collagen I mediates its effects through altered connexin-43 (Cx43) mediated hemichannel activity in human proximal tubule cells.

**Materials and methods:** Human kidney (HK-2) proximal tubule cells were cultured on collagen I (50µg/mL) and treated for 48 hours ± TGF-β1 (2-10ng/mL). Uncoated plastic served as the control. Expression of candidate proteins was determined by immunoblotting. Cell-substrate interactions were measured using the Cytoselect cell adhesion assay. Carboxyfluorescein (200µM) dye uptake and ATP biosensing were used to measure hemichannel activity and ATP release respectively in TGF-β1 treated HK-2 at 48 hours.

**Results:** Immunoblot analysis confirmed that TGF-β1 increased collagen I expression to 167±19%, 181±8% and 192±19% at 2, 4 and 10ng/mL respectively as compared to control, whilst adhesion assays confirmed that HK-2 cells exhibit increased affinity for collagen I in the presence of TGF-β1 (10ng/mL) to 0.36±0.06OD as compared to control (0.15±0.02OD) (n=4,  $P<0.05$ ). Interestingly, when cultured on collagen I, cells exhibit increased expression of Cx43 to 107±16%, with co-incubation of TGF-β1 exacerbating this effect to 136±7% as compared to control (n=5,  $P<0.001$ ). This increase in expression was paralleled by increased hemichannel activity and ATP release. Carboxyfluorescein dye uptake increased in cells cultured on collagen I ± TGF-β1 to 149±6% (collagen I) and 251±5% (collagen I + TGF-β1) respectively, as compared to control (n=5,  $P<0.001$ ), whilst biosensing confirmed a significant increase in ATP, increased from 2.2±0.4µM (collagen I) to 4.5±0.2µM (collagen I + TGF-β1) as compared to control (n=3,  $P<0.001$ ).

**Conclusion:** The current study confirms a role for collagen I in regulating connexin-43 expression, changes which are exacerbated in the presence of pro-fibrotic TGF-β1, the principal mediator of damage in the tubular region of the diabetic kidney. Increased expression of this protein, central to cell-cell communication, was paralleled by increased hemichannel mediated ATP release, most likely a consequence of diminished cell-cell adhesion and gap junction mediated intercellular communication as previously reported by the group. With studies linking increased collagen I deposition to altered cell phenotype in tubulointerstitial fibrosis, and elevated ATP release to increased fibrosis and inflammation; this study highlights a potential role for collagen I in exacerbating diabetic tubular injury by regulating connexin-mediated hemichannel activity.

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