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Trauma induces changes in cell morphology and Cx43 expression in human amniotic membrane

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Objective

Iatrogenic preterm premature rupture of the fetal membranes (PPROM) is a major complication after fetal diagnosis and surgery, leading to rupture of the membranes in 30% of cases. We developed an in vitro amniotic membrane (AM) model whereby clamped human AM is cultured in a tissue culture well while exposed to term human AF (0.5 mL) bilaterally. We examined changes in cell morphology and induction of connexin 43 (Cx43) expression after small diameter trauma in term human AM explants.

Methods

Term human AM were traumatized in vitro with a needle (1.65 mm) and cultured for up to 4 days. Cell morphology, migration and Cx43 protein expression were examined in the amniotic epithelial layer and compared to mesenchymal cells present in the fibroblast layer in AM from the placenta (PAM) or cervix (CAM). Cell morphology and Cx43 were examined in the wound edge AM by immunofluorescence (IMF) confocal microscopy with labels for F-actin (rhodamine phalloidin), nuclear (DAPI) and α-smooth muscle actin (SMA). Collagen microstructure was examined by second harmonic generation (SHG) imaging.

Results

24 hours after trauma, dense regions of mesenchymal cells had migrated to the wound edge of cultured CAM and PAM specimens. A common feature within wounded AM was the presence of mesenchymal cells that were polarized at 90° to the leading wound edge (Fig. 1). At the trauma site, we observed a dense region of highly polarized collagen fibers and a greater intensity of the SHG signal close to the wound edge in the AM and evidence of altered Cx43 expression. This collagen structure appears to be more coherent and presents a different profile to control AM where the fibre arrangement is disorganized, randomly interwoven and the cells have a rounded morphology. We observed increased F-actin, nuclear cell contraction and myofibroblasts in the wounded cultured CAM and PAM specimens 96 hours after trauma. These appear to bring the wound edges together, representing the purse string contraction model but closure was not complete.

Conclusion

Changes in Cx43 expression in the wounded AM drives changes in cell contraction, migration, collagen polarization and wound closure in small diameter membrane defects. The purse string contraction mechanism does not completely close small diameter wounds but warrants further investigation and could be an approach to repair the FM defects after iatrogenic PPROM.