Mechanical stimulation affects Cx43 and collagen integrity in human preterm amniotic membrane defects

[1]Costa E, [1]Crowther M, [2]Thrasivoulou C, Deprest JA, Becker DL, David AL, Chowdhury TT

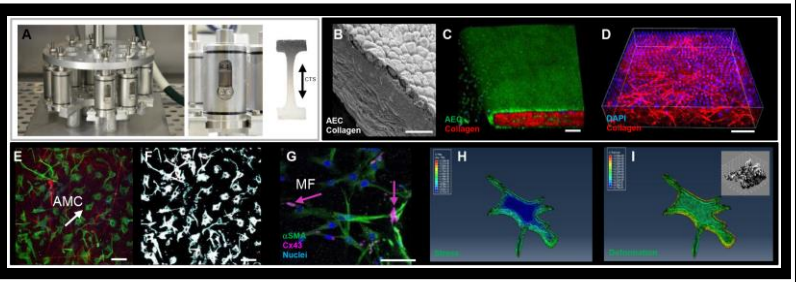
[1]Centre for Bioengineering (QMUL); [2]Department of Cell and Developmental Biology (UCL); [3]Department of Obstetrics and Gynaecology (Leuven);

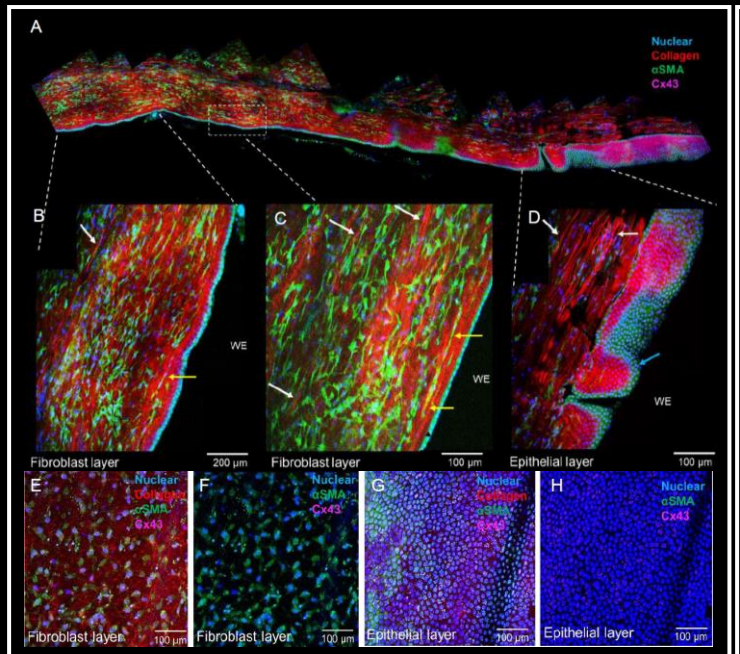
[4]Lee Kong Chian School of Medicine (NTU); [5]Institute for Women's Health (UCL)

Introduction: Human fetal membranes do not heal after fetal surgery or spontaneous rupture leading to PPROM. In term amniotic membrane (AM), we previously showed that mechanical stimulation upregulates the gap junction protein Cx43 and formed plaques between amniotic mesenchymal cells (AMCs) preventing cell migration and repair (Barrett DA *et al.*, 2016). In contrast, the highly migratory myofibroblasts expressed Cx43 and promote repair via αSMA and release of TGFβ$_1$ (Costa E *et al.*, 2021).

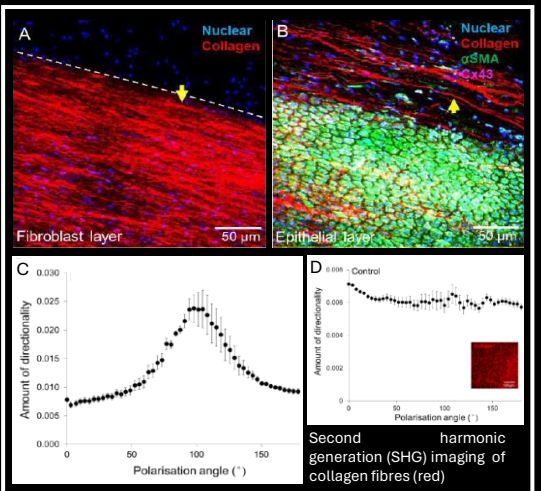
Aims: Since Cx43 is a well-established mechanoreceptor and is involved in differentiation, healing and inflammation, we examined whether mechanical stimulation activates a Cx43-stretch sensitive pathway and increases healing by myofibroblasts thereby maintaining collagen integrity in preterm AM.

Methods: Preterm classified into sPPROM (n≥32 weeks; n=3 donors; 1.5-4 cm defects). AM explants subjected to 2% CTS (A, 1 Hz, 24 hr) and compared to controls (B, no CTS). Specimens analysed by IMF confocal (C, E, G) and SHG (D). Cell shape quantified (F) for cell-scale computational model (H, I).



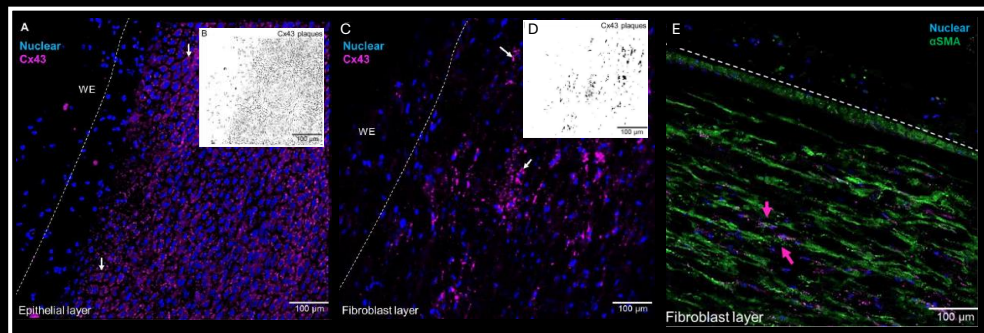


Cell morphology in preterm AM after spontaneous rupture: Myofibroblasts with cytoplasmic extensions express αSMA in wound edge (WE) specimens within the fibroblast layer (B, C). AECs migrate to the WE and formed cell clusters in the epithelial layer (D). Controls away from the WE show typical morphology of cells present in the epithelial (G, H) and fibroblast layer (E, F).



Second harmonic generation (SHG) imaging of collagen fibres (red)

Collagen organisation in preterm AM defects: In the fibroblast layer of preterm AM defects, we observed a dense region of collagen fibres parallel to the WE by SHG microscopy (A). The direction of the collagen fibres were polarised ~90-100◦ in the WE (C) in contrast to no polarity in control specimens (D). In the epithelial layer, AECs expressed αSMA and formed a layer along the wound edge (B). There was evidence of collagen degeneration and polarisation of fibres in the AM layers (yellow arrows, B).



Cx43 protein expression: Cx43 was increased by WE cells in the epithelial (A) and fibroblast layer (B). Binary images show Cx43 plaques between AECs (B) and AMCs (D) but not αSMA-expressing myofibroblasts which had cytoplasmic localisation of Cx43 (pink arrows, C).

	CTS	CTS + Trauma	CTS + Cx43as
Collagen (%)	CAM -21.5 PAM 75.6	CAM 75.6 PAM 22.0	CAM 110.3 PAM 125.7
Elastin (%)	CAM 14.8 PAM 88.3	CAM -9.4 PAM -77.9	CAM 135.6 PAM 127.9
GAG (%)	CAM 101.6 PAM 21.9	CAM 81.4 PAM 35.0	CAM 3.6 PAM 25.1
PGE_2 (%)	CAM -3.6 PAM -1.1	CAM -5.6 PAM -1.2	CAM -2.9 PAM -1.9

Preterm AM explants were traumatised with a needle to create a 0.8 mm defect and subjected to cyclic tensile strain (2% CTS) at 1 Hz frequency for 24 hr using a well-established bioreactor system (Chowdhury B et al., 2014), either in the presence or absence of Cx43 antisense (50 µM). Values represent % changes from control specimens (no stretch, no trauma), where n=6-12 replicates from 3 preterm donors n≥32 weeks.

Effect of mechanical stimulation in preterm AM defects: Mechanical stimulation differentially affects collagen and elastin levels in AM specimens close to the cervix (CAM) and placenta (PAM). The response is reduced by trauma and reversed matrix repair proteins in the presence of the Cx43 antisense. Changes in PGE_2 levels were minimal despite concentrations ~30 ng/mL.

Conclusions: In preterm AM defects, Cx43 is differentially expressed by cell types in the epithelial and fibroblast layers. Mechanical stimulation affects matrix repair proteins. Establishing how Cx43 regulates mechanotransduction could be an approach to regenerate fetal membrane defects.

Acknowledgements: This research was funded by GOSH Medical Charity (17QMU01), Rosetrees Trust (M808) and EPSRC (EP/T518086/1).