Disease-Specific Extracellular Matrix Composition Influences Placental Trophoblast Fusion

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Introduction: The placenta plays a critical regulatory role in short- and long-term maternal and fetal health during pregnancy. In humans, transport of nutrients and waste is regulated through the syncytiotrophoblast, a giant multinucleated cell formed and maintained through fusion of mononuclear cytotrophoblasts. Despite the established importance of maintaining a syncytiotrophoblast for healthy pregnancies, the *in vivo* factors regulating cytotrophoblast fusion are not well defined, and understanding these factors are critical to develop tissue engineered placental models and novel therapeutic strategies for placental disorders. We have previously established that extracellular matrix (ECM) mechanics and architecture play an important role in regulating fusion (1,2), and here, we investigate whether the composition of disease-specific ECM further regulates cell fusion.

Materials and Methods: Human placenta were decellularized and pulverized into dECM nanoparticles. Protein compositions of normal and pre-eclamptic (PE) dECM were characterized via mass spectrometry. Blank polyacrylamide gels were functionalized with either dECM or candidate ECM proteins identified through the screen. BeWo choriocarcinoma cytotrophoblasts or isolated primary villous cytotrophoblasts (vCTBs) were cultured on the surfaces under pro-fusion conditions, and fusion was assessed via E-cadherin immunostaining.

Results and Discussion: Mass spectrometry indicates considerable differences in composition between healthy and PE placentas. Both the BeWo cell line and the primary vCTBs cultured on dECM-functionalized surfaces showed no significant difference in cell attachment numbers or spread area, but significant reductions in fusion on disease-associated ECM (*Fig. 1A, B*), strongly indicating that maintenance of the *in vivo* syncytiotrophoblast is affected by disease-specific changes to matrix composition. To identify specific matrix contributions towards this phenotype, we focused on structural proteins, and noted that the ratio of Collagen I to Collagen IV increased by ~5x in PE samples. Cells cultured on surfaces functionalized with selected candidate structural proteins (Fig 1C, D) confirmed that while Collagen-IV supports fusion, replacement of this protein with other disease-related ECM components reduced fusion efficiency, which would ultimately affect maternal-fetal transport in disease states.

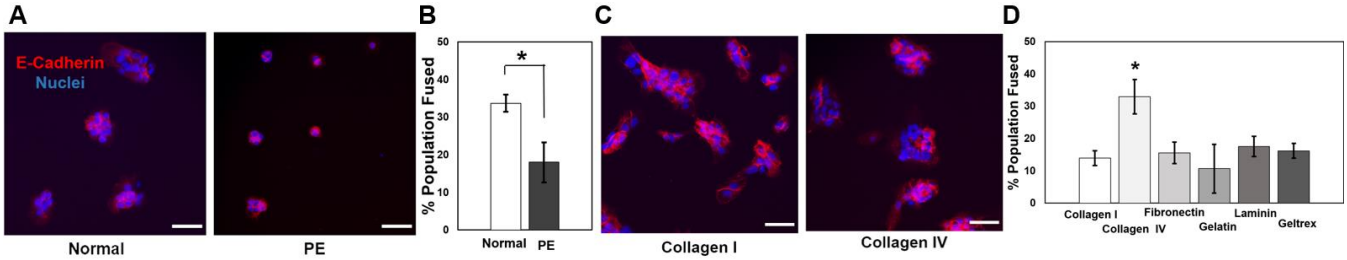

Figure 1. Representative images of fused BeWo cells on substrates containing normal dECM (A) and individual Collagen I and Collagen IV (C). (B and D) Quantification of fusion efficiency for A and C along with other proteins, respectively, showing higher fusion with normal dECM and Collagen IV. Scale bars: 100 µm; *: $p < 0.05$ (n=3).

Conclusions: Broadly, the experimental strategy developed here may be used to identify in vivo-specific effects of ECM-driven biological function. While it was previously known that transport profiles are altered in conditions such as preeclampsia(3) and that ECM protein composition changes with disease(4), this work identifies ECM-regulated fusion efficiency of the syncytiotrophoblast as a potential mechanism driving known disease phenotypes. Hence, placental ECM remodelling may be a novel target to develop pregnancy-related therapies.

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