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Supplemental Information

Characterizing Inner Pressure and Stiffness of Trophoblast and Inner Cell Mass of Blastocysts

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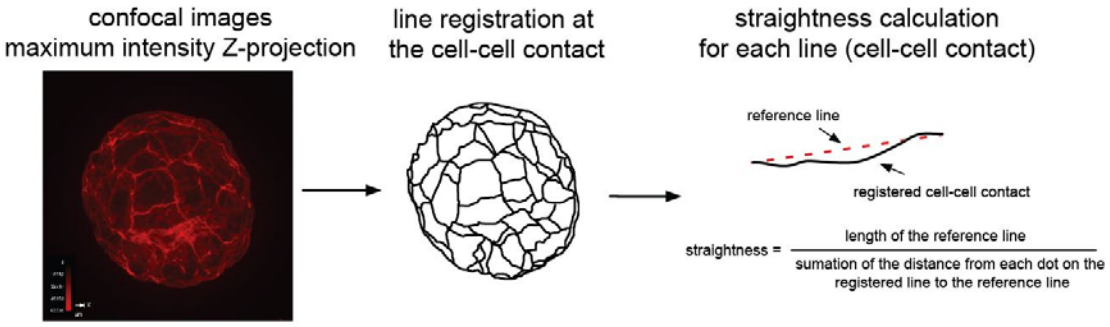

Figure S1 Line scanning for quantifying the straightness of cell-cell contact in blastocyst embryos. The confocal Z-stack images of embryos with actin labeled were first projected to X-Y plane using the maximum intensity. The image was then binarzied and the cell-cell contact was registered using Canny edge detection. The detected line was proofread manually after line detection. The straightness value was calculated as the ratio of the length of the reference line (a straight line connecting the start and the end of one cell-cell contact) over the summation of the distance from each sampled dot on the cell-cell contact to the reference line. The value of straightness is close to 1 if the cell-cell contact line is close to a straight line. A higher value of straightness indicates a higher cortical tension in the cell-cell contact. The value straightness is close to 0 if the cell-cell contact is more curved, indicating smaller cortical tension in the cell-cell contact. The average straightness value of 20 lines in each embryo was used to represent the straightnesss of the embryo.

Comparison between multi-pole magnetic tweezers and Atomic Force Microscopy

AFM can be used for measuring force-displacement data on the blastocyst surface. However, due to the barrier of the Zona Pellucida (ZP), the AFM probe cannot directly contact the trophoblast. As shown in Fig. S2(a), direct indentation onto the ZP using different indentation depths could give the Young's modulus of the ZP and trophoblast, if the first layer (ZP) is softer or similar in Young's modulus compared with the second layer (trophoblast). However, ZP is significantly stiffer than the trophoblast (~15 kPa vs. 0.3 kPa); the thickness of ZP and the gap between ZP and trophoblast varies across different stages of blastocyst, making the task of decoupling mechanical properties of the two layers (ZP and trophoblast) infeasible.

In our work, laser ablation was used to create a small access area for the microbead to directly contact the trophoblast for measurement, while creating a hole on ZP for AFM to directly access trophoblast requires the laser to pass through the embryo [Fig. S2(b)], which may undesirably create a hole on trophoblast and release the embryo inner pressure.

For the first task in our paper, i.e., decoupling embryo inner pressure and Young's modulus of trophoblast, removing all ZP was needed to enable AFM to directly contact the trophoblast for mechanical measurement. The ZP can be removed either chemically of mechanically. It is unknown whether the chemical (e.g., acidified Tyrode's solution) used for dissolving ZP would change the embryo inner pressure or mechanical properties of the trophoblast. Therefore, for the validation experiment with AFM [see Fig. 3 in the paper], we mechanically removed the ZP by conducting careful laser ablation around one focal plane of ZP, then with extreme care pulling out the embryo body using a pipette connected to the robotic micromanipulator.

This process was highly time-consuming and skill dependent. After mechanical isolation of ZP, the embryo was moved to the AFM for measuring force-displacement data [Fig. S2(c)] to validate the results from using our magnetic tweezers.

For the second task in our paper, the ICM mechanics was measured by the magnetically controlled microbead inside blastocyst of different stages. In this case, it is not realistic to use AFM for performing mechanical measurement on the ICM which is inside the embryo.

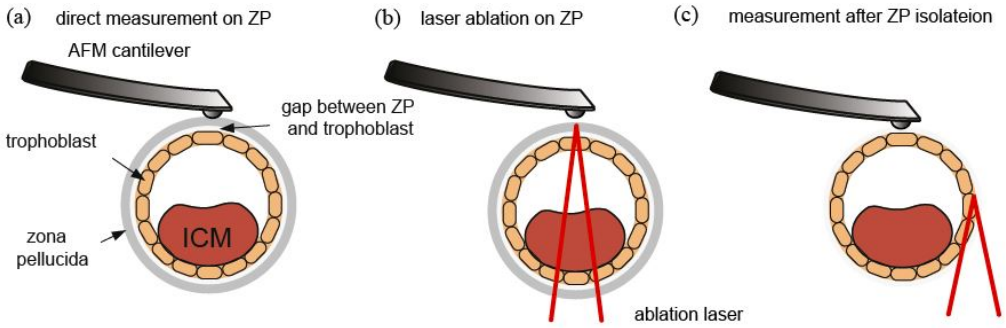

Figure S2 (a) Direct AFM measurement on zona pellucida (ZP) for decoupling the Young's modulus of both ZP and trophoblast. (b) Laser ablation on ZP to create a direct contact area between the AFM probe and the trophoblast. However, the ablation laser has to pass through the embryo, potentially disturb the embryo inner pressure. (c) AFM measurement on embryo after removing ZP. The measurement was first conducted with embryo inner pressure, then laser ablation create a hole on the trophoblast to release the inner pressure. The measurement was then conducted without the embryo inner pressure. The embryo inner pressure and Young's modulus of trophoblast can be decoupled using the force-displacement with and without inner pressure with the model Eq. (2).

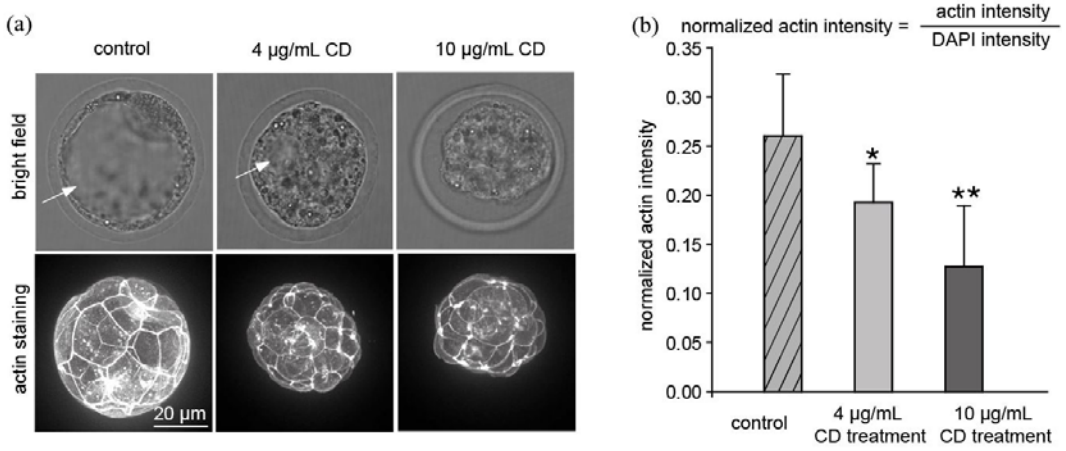

Figure S3 (a) Bright field images and actin staining after cytochalasin D treatment using concentration of 4 µg/mL and 10 µg/mL. The control group was treated with DMSO. White arrows indicate blastocyst cavity. (b) Normalized actin intensity comparision between the control group (DMSO), CD treatment of 4 µg/mL, and CD treatment of 10 µg/mL. The normalized actin intensity was calculated by the division of actin intensity and DAPI intensity. N = 6 embryos, error bar: standard deviation. *P is marginally significant, and equals to 0.056. **P = 0.004, significantlly different from the control group.