A 3D MODEL OF UTERINE FIBROID CELLULAR RESPONSE TO COMPRESSION: EXAMINING MECHANOTRANSDUCTION IN FIBROID PATHOGENESIS

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Background: Mechanotransduction, or the translation of mechanical forces into biochemical cues, is an area of limited knowledge in fibroid pathophysiology.[1] Uterine smooth muscle cells are exposed to constant mechanical forces due to uterine contractions, even in the non-pregnant uterus.[2] These contraction patterns change throughout the menstrual cycle.[3-4] Our previous research has shown that actin reorganization in response to strain is dysregulated in monolayer fibroid cell models.[5] Extracellular matrix production may also be dysregulated in fibroid cells, which may be examined by collagen content. Production of these proteins is uncommon in monolayer cultures and can be more easily studied in 3D cultures. Examining the role of compressive mechanotransduction in fibroid pathogenesis in a 3D model may provide insight into developing non-hormonal, fertility-sparing options for treatment of fibroids.

Objective: To compare the effects of compression on fibroid versus myometrial cell cytoskeleton composition, proliferation, or collagen synthesis using a 3D model.

Materials and Methods: After informed consent, cells were isolated from surgical specimen per an approved IRB protocol. Tissue spheroids were fabricated from fibroid or patient-matched myometrial cells between passages 2-4 by centrifuging aliquots of $5x10^5$ cells. After seven days, the spheroids were embedded in 1% agarose gel. Weights were added to exert 6.4 mmHg compressive force. All samples had an unweighted matched control with at least two experimental replicates. After one week of compression, spheroid area and collagen content were assessed. Image analysis compared the change in total area of the spheroids following compression. Collagen analysis was completed with Sircol insoluble collagen assay. Statistical analyses were completed with paired two-way ANOVAs with a 95% confidence interval.

Results: For spheroid area analysis, a total of 81 myometrial spheroids (n=40, 0 mmHg; n=41, 6.4 mmHg) and 70 fibroid spheroids (n=35, 0 mmHg; n=35, 6.4 mmHg) were measured from five biological replicates. All spheroids contracted when embedded within the agarose gel, as reported by percent change from original area. All spheroids had less tissue contraction in response to compressive force ($p<0.0001$). Myometrial spheroids contracted more than fibroid spheroids after seven days in culture ($p=0.0218$): 68.9 ± 8.3% (myometrial control) versus 77.6 ± 12.6% (myometrial compression), and 72.1 ± 6.9% (fibroid control) versus 82.4 ± 13.4% (fibroid compression). There was no statistically significant interaction between cell type and compression.

For collagen analysis, two spheroids were analyzed for each condition and cell type for all biological replicates. The mean collagen content for myometrial spheroids (23.6 ± 6.5 µg/mL) increased significantly following compression (25.5 ± 7.2 µg/mL, $p<0.05$), otherwise there was no significant change in total collagen content following compression after seven days.

Conclusions: Consistent with previous findings, we see differences in contraction between the fibroid and myometrial cells that affected spheroid area. Collagen content increased in response to compressive force; however, not significantly in the fibroid spheroids. Additional work is needed to understand whether differences in cell number play a role in both changes in area and collagen content, and whether longer culture times will be needed so that growth of the spheroids can be observed.

Financial Support: No outside sources of financial support

References:

1. Leppert P, Jayes F, Segars J. The extracellular matrix contributes to mechanotransduction in uterine fibroids. Obstet Gynecol Int. Published online 2014. Doi:10.1155/2014/7832389
2. Bulletti C, de Ziegler D, Polli V, Diotallevi L, Del Ferro E, Flamigni C. Uterine contractility during the menstrual cycle. Hum Reprod. 2000;15 Suppl 1:81-9. doi: 10.1093/humrep/15.suppl_1.81. PubMed PMID: 10928421.
3. Ijland MM, Evers JL, Dunselman GA, van Katwijk C, Lo CR, Hoogland HJ. Endometrial wavelike movements during the menstrual cycle. Fertil Steril. 1996;65(4):746-9. doi: 10.1016/s0015-0282(16)58207-7. PubMed PMID: 8654632.
4. Ferries-Rowe E, Corey E, Archer JS. Primary Dysmenorrhea: Diagnosis and Therapy. Obstet Gynecol. 2020;136(5):1047-58. doi: 10.1097/AOG.0000000000004096. PubMed PMID: 33030880.
5. Schutte SC, Ghosh D, Moset Zupan A, Warwar R, Dawson MR. Differential Response to Mechanical Cues in Uterine Fibroid versus Paired Myometrial Cells. Reprod Sci.Online ahead of print. doi: 10.1007/sr032-023-01267-z.