Matrix Modulus Influences the Development of Breast Acini and Ducts in 3D Cultures

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Statement of Purpose: Cells exist within a complex tissue microenvironment, which includes cytokines, diverse adhesive proteins from extracellular matrix, and physical stresses from neighboring tissues. Cells actively sense and respond to these microenvironmental cues to reach a physiological equilibrium. As investigators seek to study cells in benchtop culture systems, the selection of matrix (i.e. simulated stroma) becomes crucial. To investigate the biomechanical matrix effects on cellular organization in a breast tissue system, we co-cultured mammary gland epithelial cells and bone marrow stromal cells in a series of matrices with varying stiffness and quantified the formation of polarized acini and tubular structures.

Methods: Gelation solutions of collagen and Matrigel were mixed in equal volumes to produce gels of 0.8, 1.6, 2.4, 3.2, 4.8, 6.4 and 8mg/mL; subsequently, 250µL of each mixture was gelled in a 1.4cm circular mold. The elastic modulus of each gel was tested using atomic force microscopy (AFM). An MFD-3D-BIO™ AFM (Asylum Research) with 2.5 and 3µm radii AFM tips (spring constant 0.12 N/m) was used in this study. Each sample was tested at three points in the central area and three random points in the edge area. The edge area was defined as within 3mm from periphery of the gel. The measurements were made three times at each point. The gel elastic modulus was estimated by fitting a Hertz model to indentation depths from 800 to 1000 nm.

Hertz Model $$F = \frac{4}{3}\frac{E}{(1-\upsilon^2)} R^{\frac{1}{2}} \delta^{\frac{3}{2}}$$

F = measured force δ = indentation depth
υ = Poisson's ratio (0.5 for hydrogel) R = AFM tip radius

NMuMG (murine mammary gland epithelial cell) and D1 (murine mesenchymal stem cell) cells were co-cultured at a 30:70 ratio in serial concentrations of collagen-Matrigel gels (0.8, 1.6, 2.4, 3.2, 4.8, 6.4 and 8mg/ml). Cells organized themselves into acinus-like or tubular structures within 3 to 5 days in culture. The number of acinus-like and tubular structures was quantified using Image J software.

Results: Collagen-Matrigel matrices supported differentiation of NMuMG and D1 cells into acinus- and duct-like structures. However, the number of acinus-like and duct-like structures was altered by the matrix density/stiffness (Fig. 1). The number of acini-like structures was significantly higher in gels of 1.6 and 2.4 mg/ml; gels of 0.8mg/ml had highest number of tubular structures (Table 1).

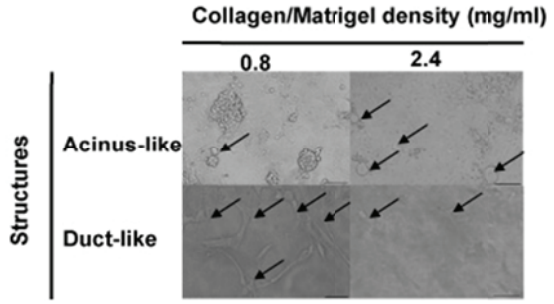

Fig 1-Representative acinus- and duct-like structures microphotographs in collagen/Matrigel (1:1 0.8 and 2.4 mg/ml). Width of each square is ~600µm; arrows point to acinus-like and duct-like structures.

The elastic modulus of collagen-Matrigel gels increased as concentration increased (Table 1). The elastic modulus was not measureable for gels of 0.8 mg/ml since the AFM tip was not able to retract completely out of the gel surface. Elastic modulus surged from gels of 4.8 to 8 mg/ml. The edge modulus and center modulus were significantly different in the 2.4 and 3.2 mg/ml gels.

Table 1-Number of acinus- and duct-like structures in collagen-Matrigel matrices

Gel	Number of Acinus–Like Structures / (mm^2)			Number of Duct-Like Structures / (mm^2)
	Central Area	Edge Area	Total	Total
0.8	N/A	N/A	3.04 ± 0.41	10.76 ± 0.6
1.6	5.56 ± 0.79	5.56 ± 1.41	5.19 ± 0.57*	5.72 ± 0.25#
2.4	3.97 ± 2.38	6.55 ± 0.87	5.19 ± 0.52*	4.13 ± 0.21#
3.2	2.38 ± 0.79	2.78 ± 0.59	2.70 ± 0.48	2.12 ± 0.21#
4.8	2.38 ± 0.79	2.58 ± 0.29	2.54 ± 0.26	3.17 ± 0.53#
6.4	0.79 ± 0.79	2.38 ± 0.67	2.06 ± 0.58	5.02 ± 0.26#
8	0.00 ± 0.00	2.38 ± 0.67	0.95 ± 0.42*	3.44 ± 0.26#

(*) and (#) represents significant difference in numbers of acinus and duct-like structures when compared to 0.8mg/mL condition (*P<0.01, #P<0.001, average ± SEM)

Table 2-Elastic modulus of collagen-Matrigel matrices

Gel	Collagen-Matrigel Concentration (mg/ml)	Center Modulus (Pa)	Edge Modulus (Pa)
0.8	0.8-0.8	N/A	N/A
1.6	1.6-1.6	25.82 ± 7.22	23.98 ± 2.41
2.4 (*)	2.4-2.4	39.16 ± 7.8	53 ± 9.68
3.2(*)	3.2-3.2	86.66 ± 3.74	107.91 ± 4.54
4.8	4.8-4.8	4203.19 ± 193.63	3941.16 ± 99.18
6.4	6.4-6.4	5439.54 ± 276.22	5453.22 ± 281.05
8	8-8	6609.90 ± 70.96	6517.46 ± 93.04

(*) represents significant difference in modulus between center and edge areas (*P<0.05, average ± SD).

Conclusions: The development of cell structures is altered simply by changing the mechanical properties of the microenvironment in which they grow. More acinus-like and duct-like structures were detected in collagen-Matrigel matrices with low modulus (0.8-2.4 mg/ml).

References: M. Swamydas et al., *In Vitro Cell Dev Biol.*, 46:673(2010).

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