# N-Cadherin mediated mechanosensitivity and its role in myocyte cytoskeleton remodeling :


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## Abstract :

Cell-matrix and cell-cell adhesions are crucial in maintaining the structural integrity and contractile function of cardiac myocytes. Changes or disruptions to these adhesions can have adverse affects on myocyte shape and cytoskeletal architecture resulting in loss in mechanical and electrical syncytium seen in heart failure. Studies in mechanobiology have focused on attachment of sub confluent cells to ECM ligands. It has not been established whether N-Cadherin (N-Cad) acts as a mechanosensor. To test the hypothesis that N-Cad is directly involved in mechanotransduction, neonatal ventricular myocytes were plated on a model gel system of varying stiffness, functionalized with N-Cad and ECM. Cells were interrogated with atomic force microscopy (AFM) and labelled for sarcomeric A -actinin and F-actin, vinculin and beta catenin. On soft (300 Pa) N-Cad gels, myocytes did not develop F-actin fibers and were devoid of sarcomeric organization. At physiological myocardial tissue stiffness (15 kPa), cells displayed striated F-actin and organized myofibrils, on stiff surfaces (60 kPa), cells displayed prominent F-actin filaments without striations and disorganized myofibrils. These changes in cellular structural and functional properties (spread area, aspect ratio, contractility as measured by AFM) as a function of stiffness suggests that cell-cell mediated adhesions are capable, at a global level, to mechanically alter myocyte function. These studies show, for the first time, that changes in N-Cad mediated traction forces can alter the cytoskeletal organization in a manner similar to integrins. These results have broad implications in understanding remodeling associated with heart failure and therapies such as mechanical ventricular assistance

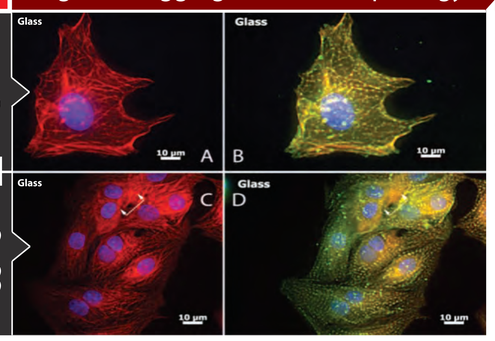

www.pages.drexel.edu/~jk45/lab/yashakresh.htm

## Background : Myocardial mechanical integrity

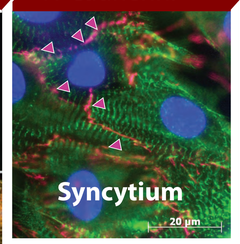
1. Cell-matrix and cell-cell adhesions are crucial in maintaining the structural integrity and contractile function of cardiac myocytes
2. Changes or disruptions to these adhesions can have adverse effects on myocyte shape and cytoskeletal architecture, resulting in loss of mechanical and electrical syncytium seen in heart failure
3. Myocardial scar is considerably stiffer than normal tissue; myocytes at its border zone experience a significantly stiffer environment which results in altered structure/morphology of these cells

## 1 Single and aggregated cell morphology :

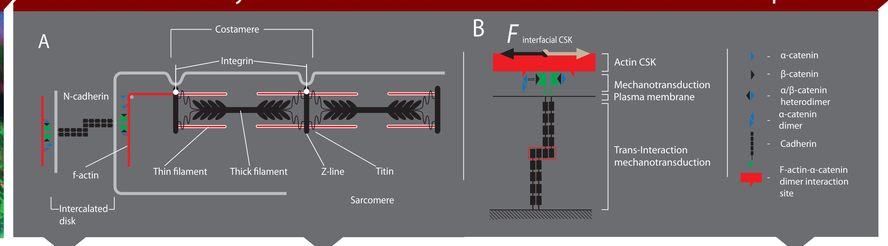
**Hypothesis :** single cell vs. aggregate




## N-Cadherin:

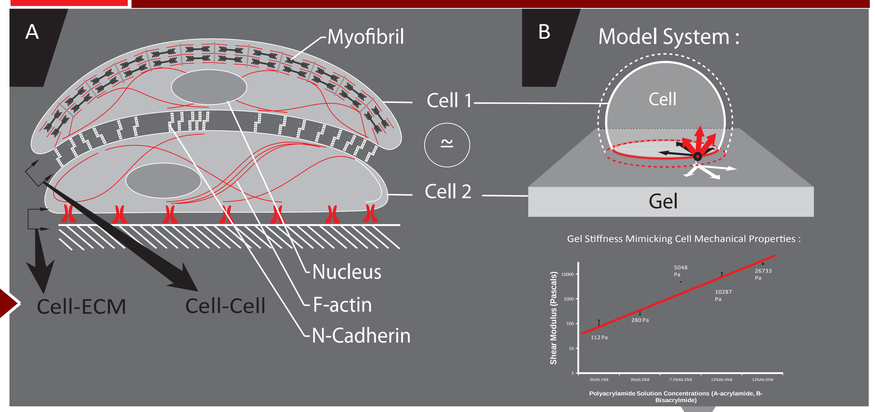


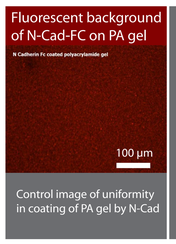

## Cells mechanically sense each other via N-Cadherin adhesive receptors :

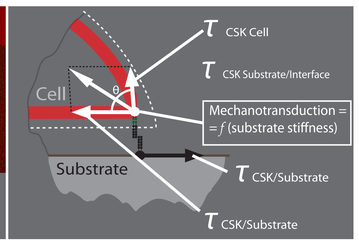



1. Single myocytes plated on stiff/soft substrates display significantly different morphology compared to that of aggregates
2. Cell-cell interactions create a mechanical continuum, transmitting forces across the cell-network
3. Cardiac myocytes express N-Cadherins within the intercalated discs, connected to the cytokeletal machinery

## 2 Cell-cell adhesion model system



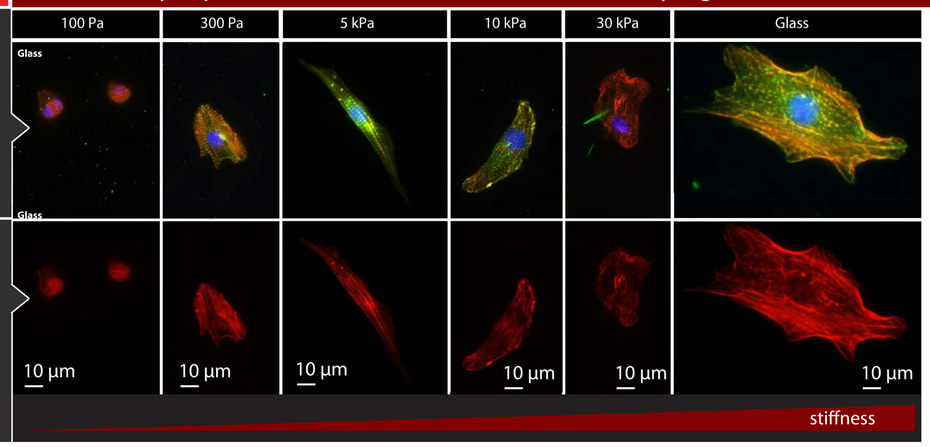




A model system to test N-Cadherin mediated mechanotransduction was created using polyacrylamide gels. The substrate mimicks the material properties of a cell ranging from soft to stiff. This model system permits the study of the effects of cell generated forces in response to substrate stiffness, mediated via N-Cadherins and/or integrins wired to the cytoskeleton

## 3 Cardiomyocytes on ECM-coated substrates of varying stiffness :

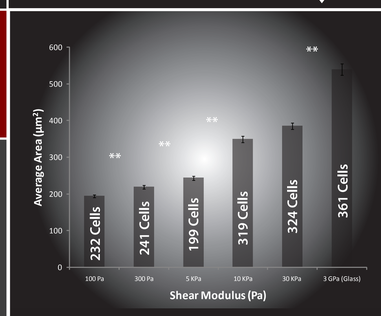
**NVRM on ECM :** α-actinin + f-actin / f-actin



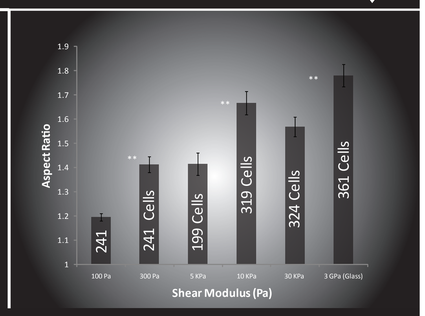

**Single cardiomyocytes on ECM -coated substrates of varying stiffness display differential morphology indicating that cells generate varying forces via integrins-mediated adhesion**

- Neonatal ventricular myocytes (NVRM) were plated (48 hr) on ECM-coated polyacrylamide gels of varying stiffness
- On soft gels (shear modulus G~100Pa) cells exhibit a rounded morphology, with poor sarcomere organization
- At physiological stiffness (G~5KPa, 10KPa), cells exhibit striated F-actin and α-sarcomeric actinin fibers, increased spread area while maintaining a high aspect ratio
- On stiff surface (G~30KPa, Glass), cells exhibit prominent F-actin filaments devoid of striations, consistent with stress-fiber like appearance

### ECM Average Area




### ECM Average Aspect Ratio

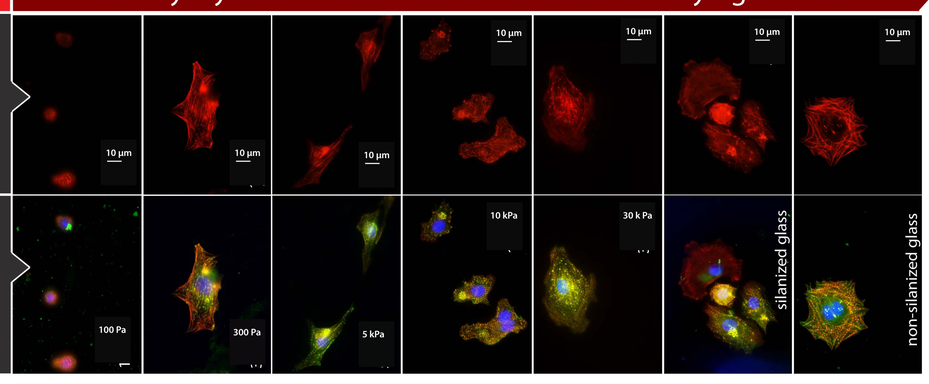



Left: Comparative bar graph of cell spreading area on varying ECM matrix stiffness reveal an increase in spread area as a function of increasing stiffness.

Right: Aspect ratio of cardiac-myocytes responsive to varying stiffness. ** indicate a P value <0.05 * indicate a P value <0.01

## 4 Cardiomyocytes on N-Cad-coated substrates of varying stiffness :

**NVRM on N-Cadherin :** f-actin / α-actinin + f-actin




**Single cardiomyocytes on N-Cad-coated substrates of varying stiffness display differential morphology indicating that cells generate varying forces via N-Cad-mediated adhesion**

- Neonatal ventricular myocytes (NVRM) were plated (48 hr) on N-Cad-coated polyacrylamide gels of varying stiffness
- On soft gels (shear modulus G~100Pa) cells exhibit a rounded morphology, with poor sarcomere organization
- At physiological stiffness (G~5KPa, 10KPa), cells exhibit striated F-actin and α-sarcomeric actinin fibers, increased spread area while maintaining a high aspect ratio
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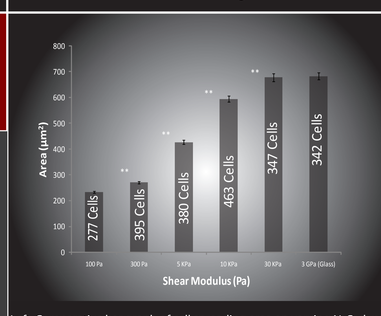
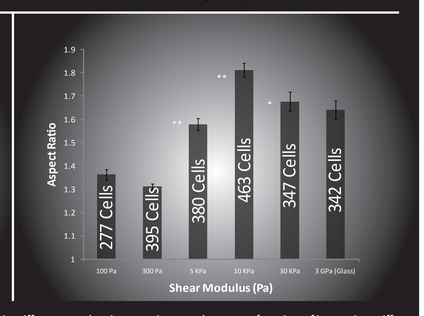
### N-Cadherin Average Area




### N-Cad Average Aspect Ratio

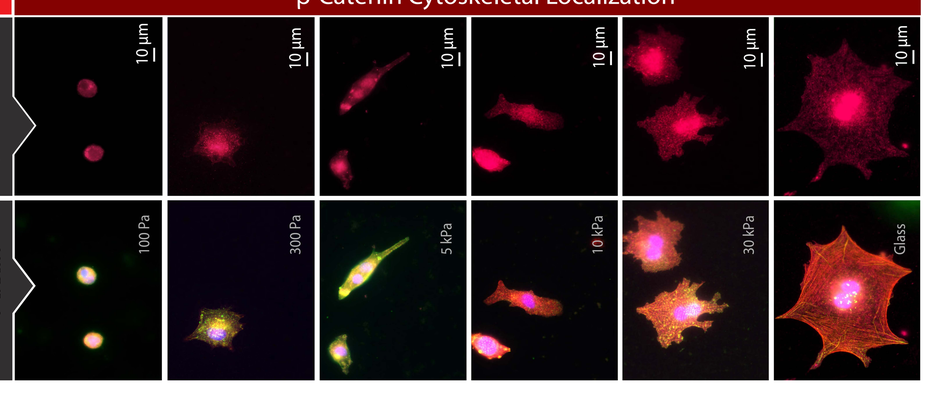



Left: Comparative bar graph of cell spreading area on varying N-Cad matrix stiffness reveal an increase in spread area as a function of increasing stiffness.

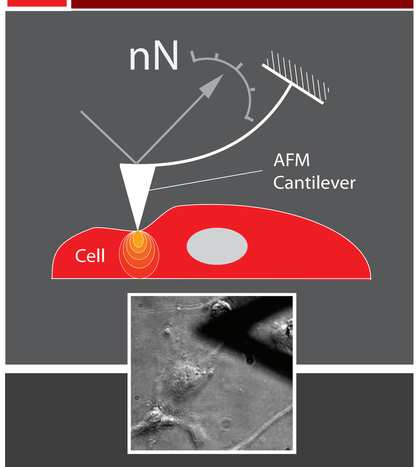
Right: Aspect ratio of cardiac-myocytes responsive to varying stiffness. * indicate a P value <0.05 **indicate a P value <0.01

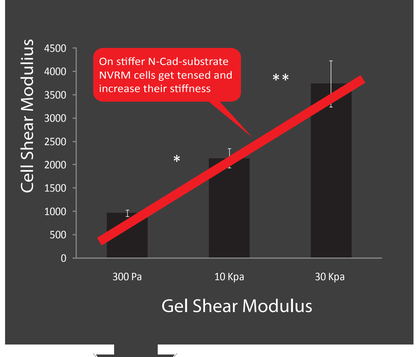
## 5 β-Catenin Cytoskeletal Localization

**NVRM on N-Cad :** β-catenin / f-actin




## 6 Cell Stiffness Measurment by AFM on N-Cad-Coated Gels




**AFM**

Atomic Force Microscopy measurments indicate that cell stiffen in response to the imposed stiffness of the adhesion surface

## Conclusion :

1. Changes in cellular structural and functional properties (spread area, aspect ratio, stiffness modulus as measured by AFM) in response to imposed substrate stiffness show that cell-cell mediated adhesions are capable at a macro-level to mechanically alter myocyte behavior
2. These studies show, for the first time, that N-Cad-mediated structural response to the mechanical microenvironement can alter the cytoskeletal organization in a manner similar to integrins
3. These results have broad implications to the understanding of remodeling-associated with heart failure and therapies such as surgical reshaping and mechanical ventricular assistance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