Single molecule force spectroscopy to determine specific receptor densities on live cancer cells under varying conditions

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Introduction

Despite recent advances in treating cancer, cancer is still a major cause of human mortality. There are still many unknowns in cancer progression, including the detailed response of cancer cells to their environment and how cancer cells modify their surrounding environment.

Most human cancer show irregular function of one or more receptor tyrosine kinases (RTKs). Collected evidence hint that a unique set of RTKs known as Discoidin Domain Receptors (DDRs) play vital role in progression of cancer by regulating the interaction between cancer cell and surrounding collagen matrix [1, 2, 3]. As a collagen sensor, DDRs control and regulate the signals from cell matrix interaction and hence cell polarity, cell differentiation. In cancer, DDRs are hijacked by cancer cells thereby disturbing the regular communication between cell and matrix [3]. Hence it would be helpful to know the interaction of DDRs with collagen matrix in the study of cancer.

- Single-molecular measurement procedures help us to uncover molecular heterogeneity.
- The single-molecule technique is based on a kinetic approach that can be carried out in natural environments even when the concentration of molecules is very low [4].
- AFM is a useful technique as it cannot just image the sample with very high resolution, but also measure the force required to break the bonds between proteins and ligands [5].

DDRs:

- Receptor tyrosine kinases (RTKs) are the high affinity cell surface receptors for many polypeptide growth factors, cytokines, and hormones [6].
- Receptor tyrosine kinases are not only key governors of cellular processes but also to have a vital role in the growth and progression of many kind of cancer [7].
- There are 6 different members in receptor family, and one of them is discoidin domain receptor (DDR) [8].
- DDRS are unique RTKs in a sense that they bind to collagens [1].

Collagens:

- Collagen is composed of a triple helix structure that contains three left-handed chains twisted together to form a superhelix.
- Collagen is found in 28 different structures [8], however, we will be focusing on type I collagen found in bone, tendon, organs, skin and type IV collagen found in hair, placenta.

Using single molecular force spectroscopy, we are measuring the rupture force of the bond between DDRs and collagen. Analyzing the data obtained we are able to extract parameters like the rupture force (bond strength), the activation barrier, binding probabilities, bond length, off-rate, and the receptor density.

Survival probability of a bond:

$$\frac{dS(t)}{dt} = -K_{off}(t)S(t)$$

Bond breaking probability at certain force f:

$$P(f) = \frac{K_{off}}{\dot{f}}\, exp\left(-\frac{\int_0^f K_{off}(f')df'}{\dot{f}'}\right)$$

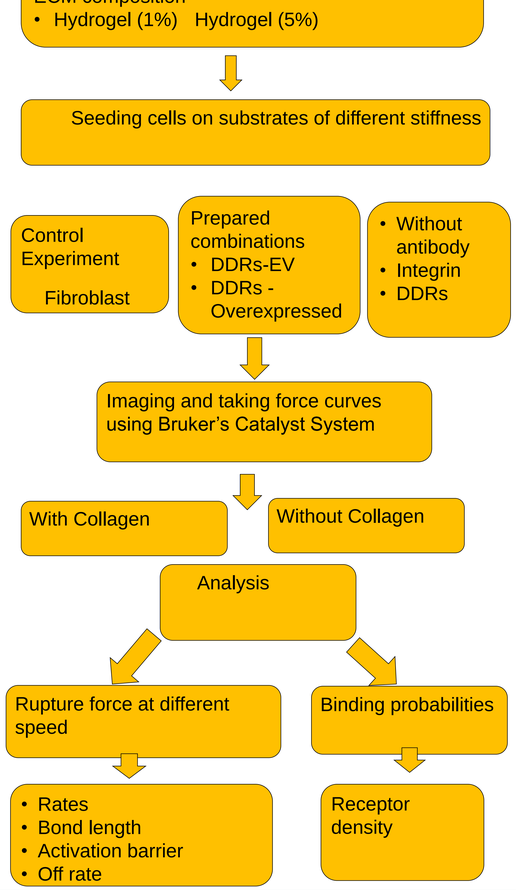
Off rate: Off rate at zero applied force:

$$K_{off} = \tau_D^{-1}\, exp\left(\frac{-E_b^o - fx^*}{K_BT}\right) \qquad K_{off}^o = \tau_D^{-1}\, exp\left(\frac{-E_b^o}{K_BT}\right)$$

We can solve following equations to find the binding probabilities due to specific receptors (DDR or Integrin):

$$\begin{aligned} p_k &= p_{D,k} + p_{I,k} \\ p_{k,Dab} &= \alpha p_{D,k} + p_{I,k} \\ p_{k,Iab} &= p_{D,k} + \beta p_{I,k} \end{aligned} \quad *$$

Methods



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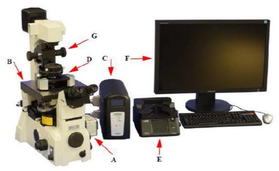
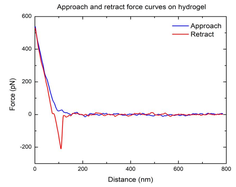


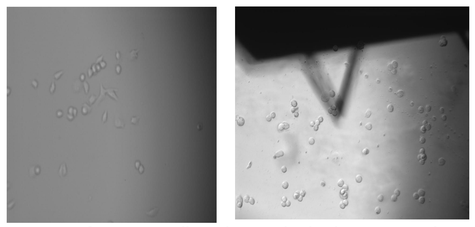
AFM setup

A- Inverted Optical Microscope
B- Bioscope base plate
C- Electronic system box
D- AFM head
E- Easyalign
F- Computer Monitor
G- Condenser

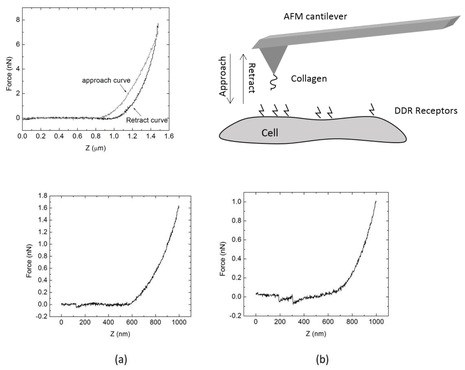
Results

Substrate stiffness:
Stiffness of the HA hydrogels prepared in the lab using Low MOD MEHA, Irgacure (I2959) by UV crosslinking was measured using Bruker's Catalyst System. A DNP-10 cantilever with spring constant 0.075 N/m was used and the observed force curves were fitted using Hertzian model to calculate the stiffness of the hydrogels.
The elastic moduli of the hydrogels (1, 2, 5 % HA Stock concentration) Were measured to be 8.33 kPa, 11.6 kPa and 19 Pa.





Images of cancer cells using optical microscope along with cantilever



Upper right: Schematic of measurement. Upper left: Undecorated tip. Lower left: Tip decorated with collagen, single rupture event. Lower right: Tip decorated with collagen, multiple rupture event.

Results

Measured binding probabilities on various cell types. Binding probabilities are due to binding to DDRs and integrin. By solving equations (*) for various conditions, such as using blocking antibodies, these can be separated.

Experimental conditions	$BPH1_{scr}$ cells	Parental $BPH1_{shDDR1}$ cells	MiaPaCa-2 EV cells	MiaPaCa-2 DDR1b cells	MiaPaCa-2 DDR1b R105A cells
No blocking agents	0.53	0.27	0.12	0.41	0.14
With integrin blocking antibody	0.28	0.04	0.02	0.20	0
With DDR1 blocking antibody	0.29	0.23			

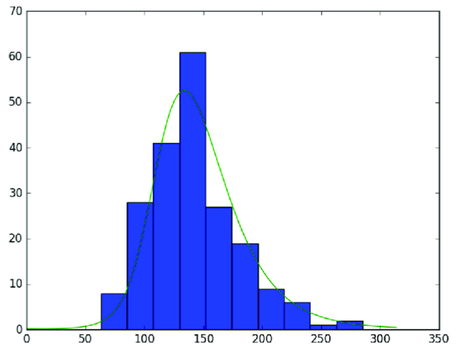
Dissociation (off-) rate and bonding distance determined from maximum likelihood fits of the force histograms.

Parameter/receptor	$\log K_{off}$	K_{off}	x*
DDR1	-1.97 ± 0.29	$0.011_{-0.006}{}^{+0.009}$ S^{-1}	0.30 ± 0.03 nm
β1-Integrin	-2.25 ± 0.20	$0.006_{-0.002}{}^{+0.003}$ S^{-1}	0.35 ± 0.02 nm

Receptor densities determined from binding probabilities, linker lengths, tip dwell time and tip speeds:

Cell type/treatment	$BPH1_{scr}$	$BPH1_{shDDR1}$	MiaPaCa-2 EV	MiaPaCa-2 DDR1b	MiaPaCa-2 DDR1b R105A
No blocking antibodies	θ = 68 μm^{-2} N = 9.9×10^4	θ = 10 μm^{-2} N = 1.5×10^4	θ = 5 μm^{-2} N = 7×10^3	θ = 49 μm^{-2} N = 7×10^4	θ = 0 μm^{-2} N = 0

Estimates of active DDR1 densities and total number of receptors per cell (assuming 25 μm diameter, 6 μm height) for the two cell types and their variants.



Maximum likelihood fit (line) to a measured rupture force histogram for $BPH1_{shDDR1}$ cells in the presence of DDR1-neutralizing antibody. Using the most probable rupture force, we calculated activation barrier, bond length, and rates.

Conclusions

- We were able to show the efficacy of using AFM in conjunction with biomedical methods to determine the kinetics and density of receptors on live cancer cells.
- We were able to isolate interactions between DDR1-collagen I on live cells excluding others (integrins).
- We measured parameters such as dissociation rates, bond length, density of receptors using the measured rupture force and binding probabilities.

Future works

- We are now beginning wok to refine and use these methods on pancreatic cancer cell lines.
- We can combine fluorescence and AFM in future experiments on live cells
- We will work on developing a method which better deals with cell membrane deformation while performing single molecule force measurements on live cells.
- We would like to perform speed-dependent force measurements of DDR1-collagen interactions
- We would like to explore effects of linkers on forces using various kinds of PEG linkers

Acknowledgement

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