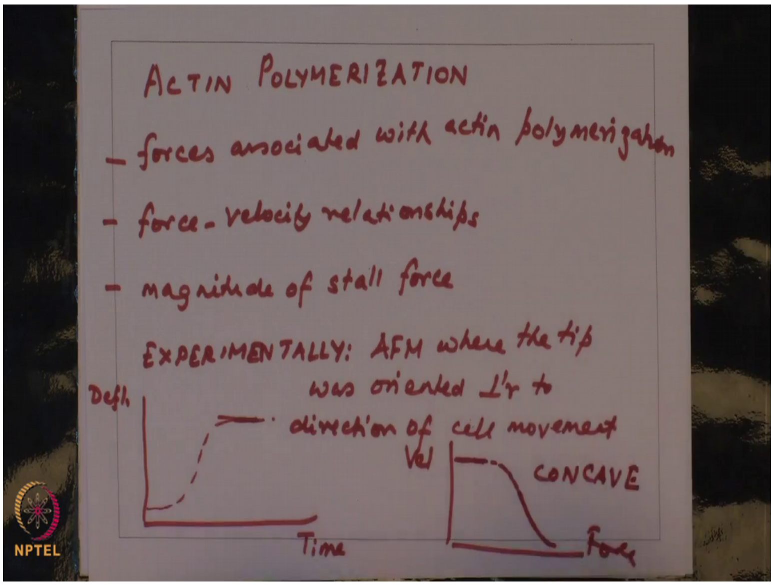
Introduction to Mechanobiology
Prof. Shamik Sen
Department of Bioscience & Bioengineering
Indian Institute of Technology, Bombay

Week - 04
Lecture - 17
Force-velocity relationships of actin networks

Hello and welcome to our 17 lecture of NPTEL course introduction to mechanobiology. So, in the last class we were discussing about dynamics of actin growth right.

(Refer Slide Time: 00:27)

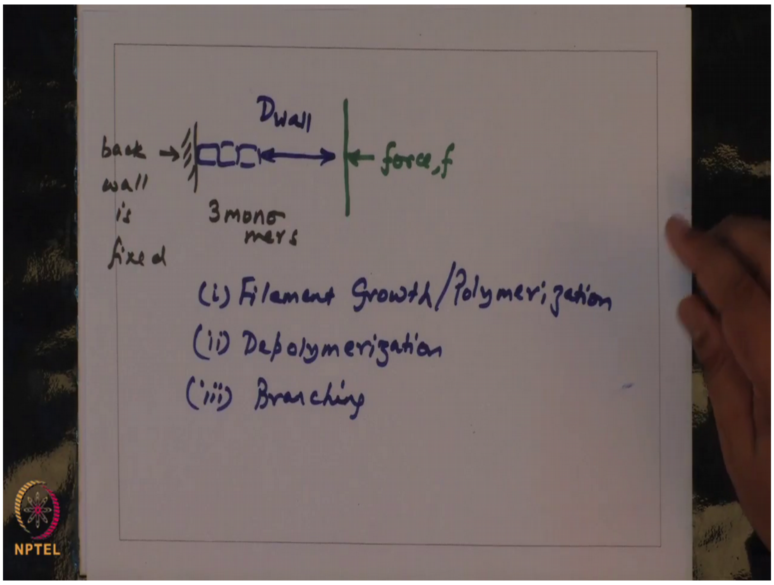


So, we are talking about actin polymerization and we trying to understand how can we quantify the forces associated with actin polymerization and the nature of force velocity relationships, and the calculating the magnitude of stall force. So, for calculating stall force experimentally, I had given an example where we use an AFM where the probe the tip was oriented perpendicular to direction of cell movement ok.

So, and as a consequence you can track the deflection as a function of time, and you would get a deflection profile which is something like this and the force. So, once it plateaus; that means, from this curve we can get the speed or velocity and the force as well. So, velocity is nothing, but slope of this curve and force is k times deflection where k is the spring constant of the tip. So, with this what the authors found was you had a force velocity curve, which looks something like this. So, it is concave its nature the concave force velocity curve.

So, and we wanted to ask that can we develop a modeling formalism for testing how various parameters contribute to dictating the nature of force velocity relationships.

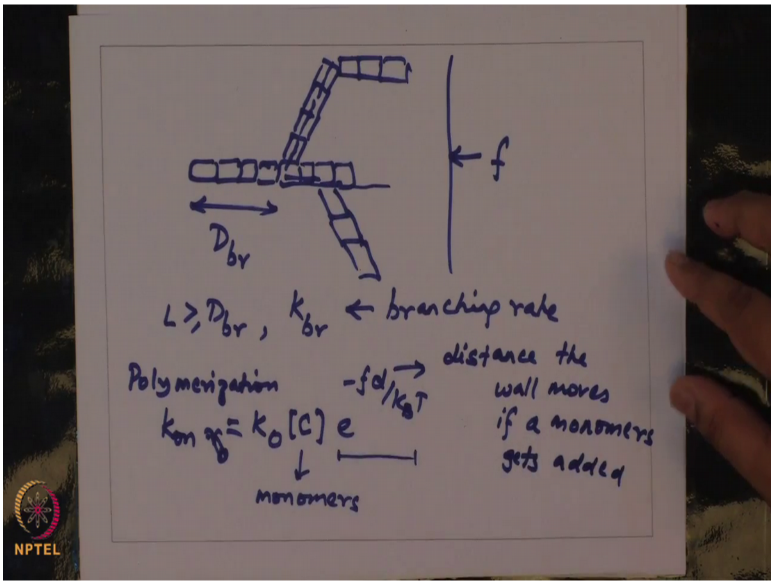
(Refer Slide Time: 02:57)



So, in that regard I had introduced the simple model where you have individual monomers, which is constraints. So, you have you start with a filament of three monomers. So, this back wall is fixed and this filament polymerizes against a wall which is on which you exert a force f, and you want to see; what is the network dynamics of this growth, for different values of branching rate so on and so forth ok.

So, the events that we said we discussed are possible is filament growth or polymerization depolymerization and branching. So, let us assume the initial distance of the filament from the wall this distance is d of wall and what you have another length scale which is that if you have a filament of certain.

(Refer Slide Time: 04:33)

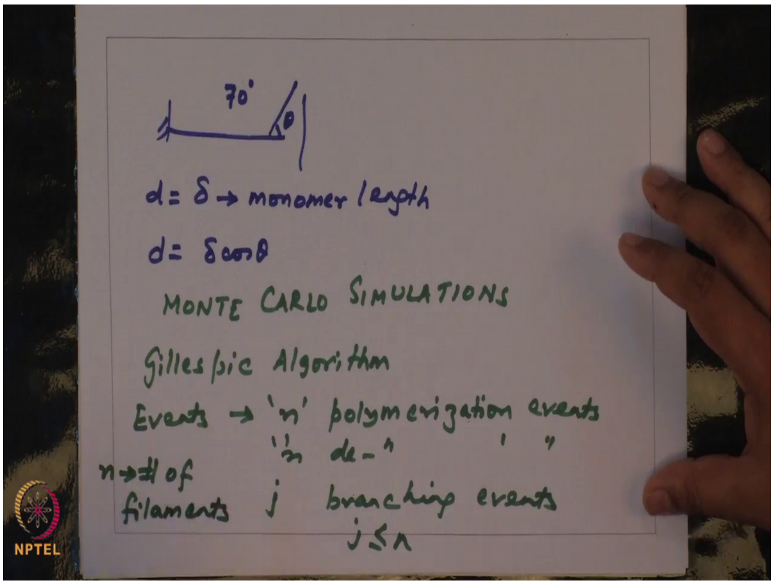


So, you need a minimum distance of Dbr before a new filament can form and branch from this point. So, in other words this is the minimum distance which means that for any filament length L greater than Dbr or greater equal to Dbr, you have a finite probability of or you have a finite branching rate. So, it is not necessary that as soon as an existing filament becomes Dbr reaches a length of Dbr, the next filament has to branch.

So, as the consequence of this you can have multiple branching events. So, if my Dbr in this case is 4 monomers, once I have 4 monomers I can theoretically form another filament which grows horizontally so on and so forth. Similarly from here for the existing filament to grow I can have another situation where a filament branches like this. So, you have a branching rate of Kbr which is the branching rate. So, the polymerization rate can be given by r on. So, if K0 is the intrinsic polymerization rate you have a concentration c of the monomers and exponential minus f d by KBT is the k on. So, this is the polymerization rate.

So, what we have here is an exponential dependence on force f, which is with which the wall is being restrained and d, d correspondings to the distance the wall moves if a monomer gets added.

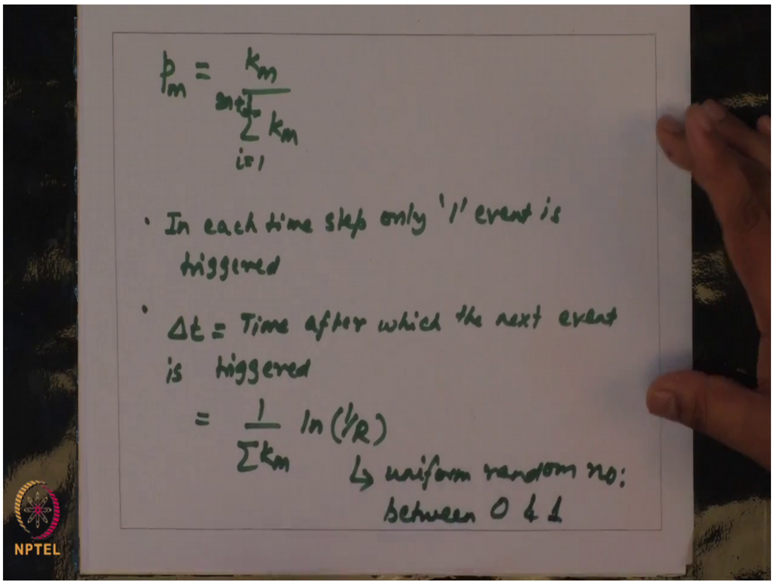
(Refer Slide Time: 07:04)



So, for this case of a straight filament let us say your d is nothing, but equal to delta where the delta represents the monomer length, but if you have at an angle, then d is equal to delta cos theta. So, this angle theta is 70 degrees.

So, we want to study the growth of this actin network, for that we use Monte Carlo simulations and specifically we use an algorithm called Gillespie algorithm. So, in Gillespies algorithm what you do is. So, you have the total number of events. So, let us say what are the events you have total number of events. As n polymerization events, n de polymerization events and j branching events, where j is less or equal to n where n responds. So, n is the number of filaments ok.

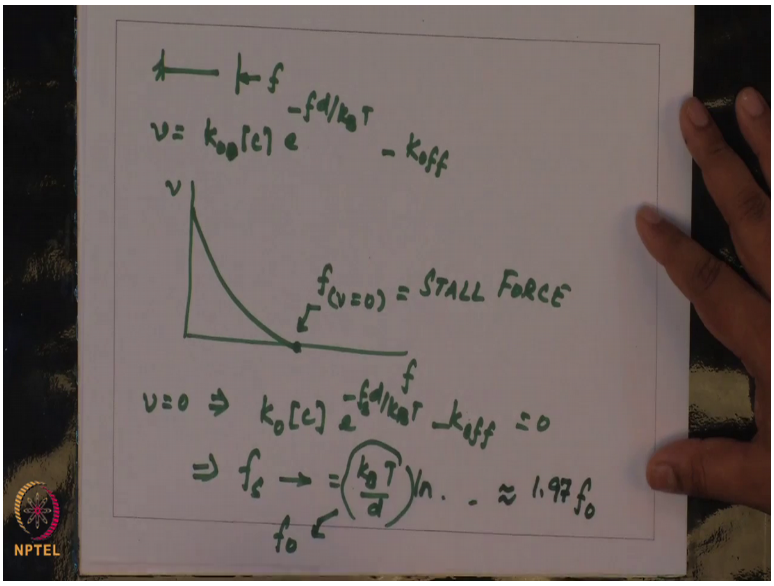
(Refer Slide Time: 08:46)



So, I can calculate the probability of an event pm as km by summation of km over i equal to 1 to 2 n plus j. So, given this what I do is in Gillespie algorithm in each time step only one event is triggered and the time after which the next event is triggered delta t equal to is given by 1 by summation of all rates into log of 1 by R where R is a uniform random number between 0 and 1.

So, using this ok; so let us see what we can simulate ok.

(Refer Slide Time: 10:12)

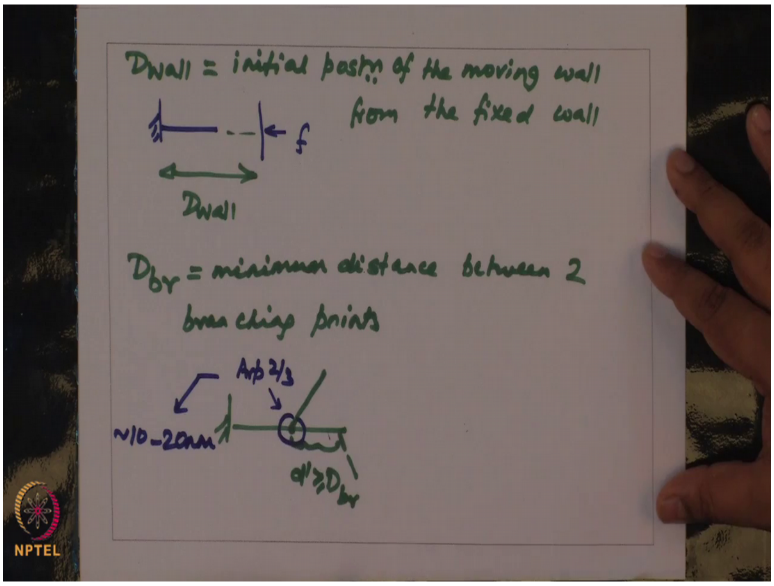


So, let us begin by case in for the case of a single filament which is polymerizing against a wall subjected to a force f and this filament is horizontal; so, for I can write the expression for speed or velocity of the filament, a velocity of filament growth as K on c. So,K on is K0 into c into e to the power minus fd by Kt minus K off. So, once we have this particular expression. So, this is the velocity right.

So, stall force I can determine in the stall force for this particular. So, first of all; so the velocity profile will look something like this exponential right you have an e to the power minus fa fd term. So, this particular value at which v is 0. So, f corresponding to v 0 is called the stall force. So, I can set v equal to 0 in this expression and I can have k 0 concentration of monomers e to the power minus fd fs d by KBT minus K off equal to 0.

So, from here I can find out the value of fs. So, this will turn out to be KBT by d exponential. So, you have k off by k naught c into log of something. So, this is some intrinsic force scale you can call it f naught. So, if you plug in the values for an actin monomer system you will get fs of the order of 1.97 f naught.

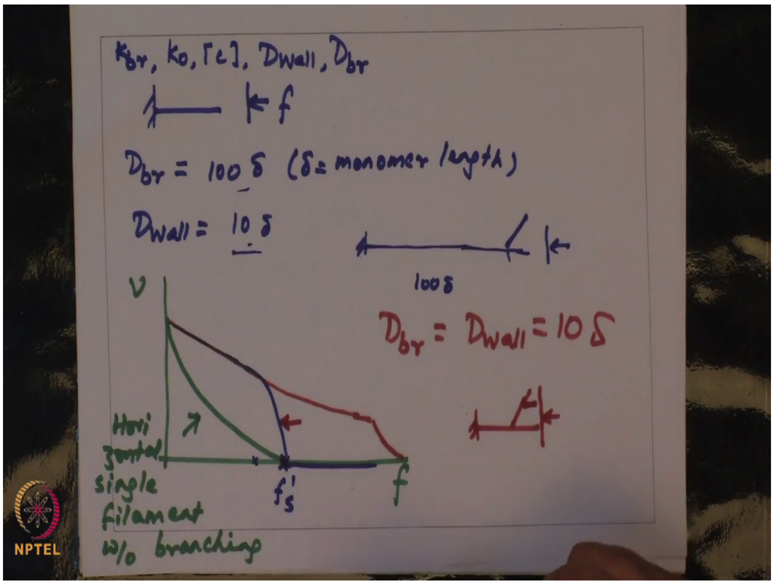
(Refer Slide Time: 12:40)



So, now let us see how will the force velocity relationship look for a network. So, let us say. So, you have two length scales here one is D wall, which is initial position of the moving wall from the fixed wall. So, this distance is D wall. So, I think earlier I had a representative this distance is D wall you can choose either way because you know that this is the length of initial length of the filament is three monomers. So, it does not matter.

And what we have another length scale is Dbr. So, Dbr is the minimum distance between two branching points. In other words if I have a filament going like this and a filament has branched at this point there is a minimum distance beyond which another filament can branch from here. So, this distance d prime has to be greater or equal to Dbr and what is the physical significance of that? One possible reason is this branching event is mediated by a protein called Arp 2 3 and the size of Arp 2 3 has been estimated to be all the other of 10 to 20 nanometers.

(Refer Slide Time: 14:46)



So, if we have a big protein sticking to this point there is a likelihood that the next binding zone will be some distance away from this binding point. So, you have Dbr and D wall. So, now, imagine now imagine the simple case you have this system you have one rate of branching rate you know K 0 c everything is given D wall is given and Dbr is given. So, let us assume Dbr as 100 times delta where delta is monomer length and D wall is 10 delta. So, what this suggests is that the wall is only 10 delta away from the back wall, while it takes the filament has to be 100 delta in length before a new branch can form ok.

So, if I look at the velocity versus force curve for the single filament. So, this is corresponding to horizontal filament single filament without branching. So, it is obvious or one would anticipate that if I operate if my stall force let us say this restraining force. So, this is fs corresponding to a single filament this value. Now imagine I am operating with a force value equal to the stall force of a single filament, at this force it is unlikely that the filament will grow to reach a length of 100 delta ok.

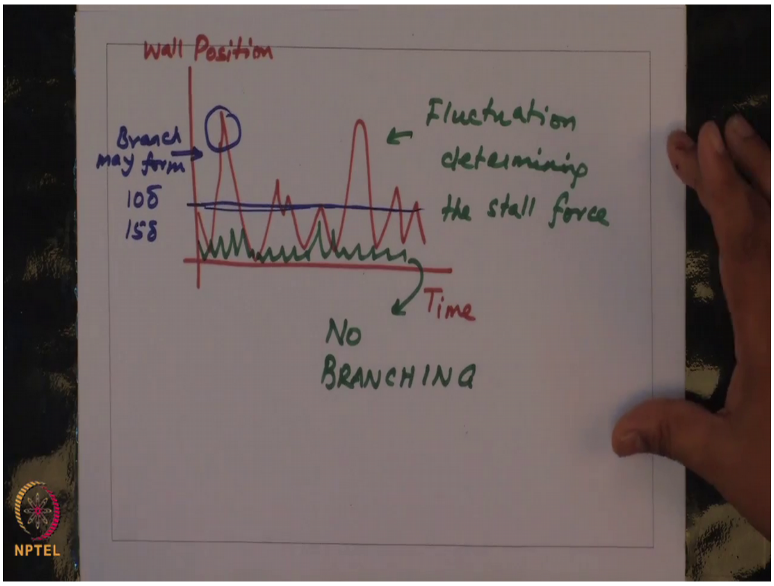
So, the value for this curve for this system here you will have the same value of the stall force and beyond also it will be 0, but imagine that if the force on the wall is less than the stall force let us say we have operating somewhere here at this value of force. So, there is a possibility that a single filament will keep pushing the wall further and further, till you reach a distance of a 100 delta such that a new filament can form and once the new filament is formed you have 2 filaments pushing against the wall. So, the stall force of that network is expected to increase ok.

So, you will realize that this will have a curve something like. So, you have a force curve for a single starting from a single filament, if you have one branching event you will begin to see for Dbr equal to 10 delta, Dbr equal to 100 delta and D wall equal to 10 delta you will get a divergence of the force velocity curve from this single filament case. Now let us assume I have Dbr equal to D wall equal to 10 delta what kind of a force curve may I expect.

So, in this case the branching distance is exactly the same as the wall distance, now given this particular phenomena that we just discussed you are almost sure that the that a new filament will branch, in that case it turns out your you will have a force velocity profile it will start from here and will have a force velocity profile like this. So, the presence of branching or the occurrence of branching really shifts the stall force to higher and higher forces, because you have systems. So, you can have various geometries let us say one geometries this kind of case, where you have two filaments prelim. In this case if you have a third filament which forms then again the stall force will increase.

So, this suggests now let us see why is it for back to the this particular case ok.

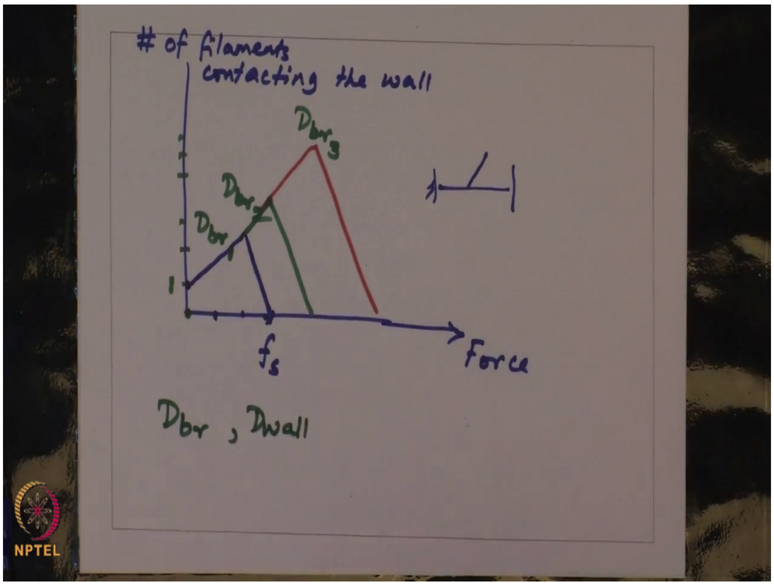
(Refer Slide Time: 19:27)



Where Dbr equal to 100 delta and D wall equal to 10 delta how did we deviate from this particular case? So, to understand that if you plot the position of the wall, as a function of time; so, if you plot it as a function of time you will see these wide fluctuations are there and let us assume that this length is equal to either 10 delta, in that case multiple number of times 10 delta or 15 delta you will see every once in a while the position of the wall is much beyond this distance at which branching is figured.

So, once every time for these events then the wall position gets pushed beyond the branching distance there is a new branching event branch will form or may form because you have a finite probability. And once the branch is formed the stall force will increase because now there are two filaments which push against the wall. So, in this case if you were to completely suppress these fluctuation dependent phenomena. So, this is an example of fluctuation determining the stall force. So, if you were to completely stop any growth of filaments or branching, you must ensure that the fluctuation for one particular case even with the fluctuation it always remains below this particular case.

(Refer Slide Time: 21:35)

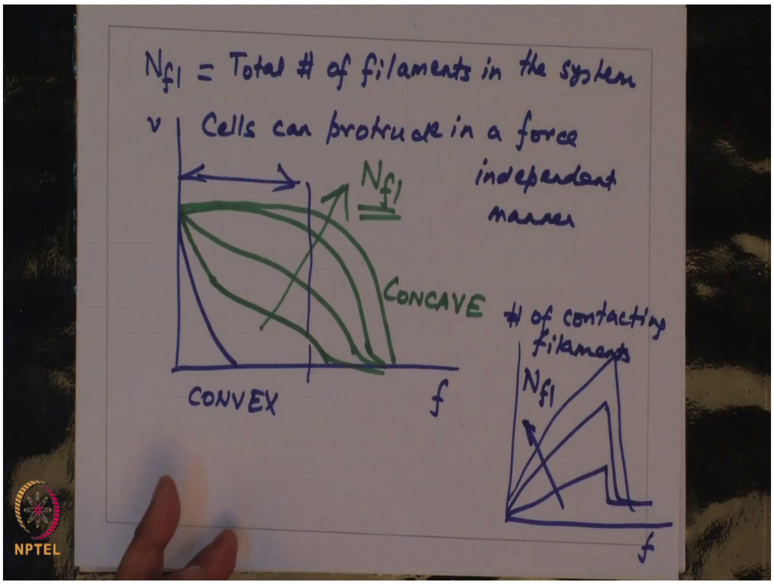


So, in this event there will be no branching, because even with fluctuation you do not surpass the branching distance Dbr. So, you can also understand this effect of fluctuation by plotting the number of filaments contacting the wall as a function of force. So, let us say if my force was 0 this is a value of one. If my force for 0 I am starting from one filament and because there is a single filament which can grow against 0 force the filament will keep on pushing the wall more and more. So, even if you whatever your value of branching distance under 0 force even if you have a branch getting created, the branch will never get in touch with the wall ok.

So, the number of contacting filaments will remain one, but as you increase the; you know the force you will see an increase in the number of contacting filaments, because this filament even if it pushes the wall it is, it will push so slowly that the branching filament can actually grow under no force conditions and then catch up with the horizontal filament. So, eventually for every case you will see a drop. So, this corresponds to the stall force for another case. So, as you increase the branching distance you will see that these curves will keep on going up and up.

So, this is let us say lowest Dbr value Dbr 1, Dbr 2, Dbr 3. So, if you use the smallest value of Dbr. So, as you increase the values of Dbr your curves will shift up and up. So, you see how this system can change and how the stall force of the system is determined by the branching architecture of the network and which in turn is dependent on Dbr and D wall. So, what will be the growth profile of this network imagine that I have I constrained the total number of filaments in the system N filament is the total number of filaments in the system ok.

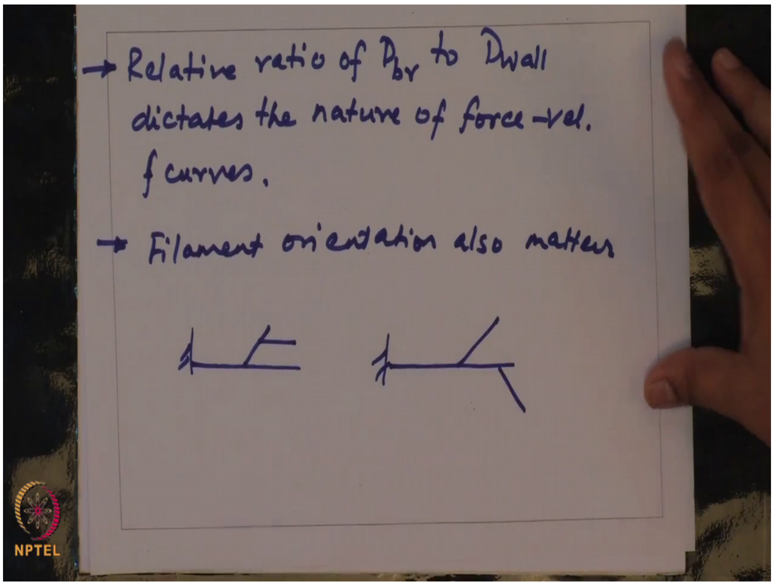
(Refer Slide Time: 24:00)



So, what you will see the force velocity curve what you will see. So, this is again the stall force for a single filament, what you will see is as you increase the stall force let me colour it as you increase the number of filaments. So, not the stall force the number of filaments you will have curves which look like this initially and eventually this direction you are increasing the number of filaments. So, from the profile you see that as you increase the number of filaments, beyond a certain value of Nfl your curve is concave. So, right most end is concave left most end is convex. Increasing the number of filaments will make the force velocity curve more and more concave, which means over a wide range of forces in this range cells can protrude in a force independent manner ok.

So, when you increase the number of filaments in the system the number of contacting filaments as a function of force. So, this is with increasing in the value of Nfl. So, increasing the number of filaments will lead to increase in the number of you know number of contacting filaments also, but beyond a stall force you see there is a sharp drop. So, because the first filament itself would not increase would not branch. So, with that I end here.

(Refer Slide Time: 26:41)



So, just to conclude there are two important messages relative ratio of Dbr to D wall dictates the nature of force velocity curves, this is one. The second one is filament orientation also matters. Because even if we have only three filaments in the system this is one particular geometry versus this might be another geometry. So, there will be difference in the nature of the force velocity curve, depending on what is the geometry of the filaments.

I thank you for your attention.