Drug Delivery Principles and Engineering
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Lecture – 42
Research Paper Discussion Dry Powder Particle Delivery

Hello everyone, welcome to another lecture for Drug Delivery Engineering and Principles; in this class we will discuss a paper on inhalation.

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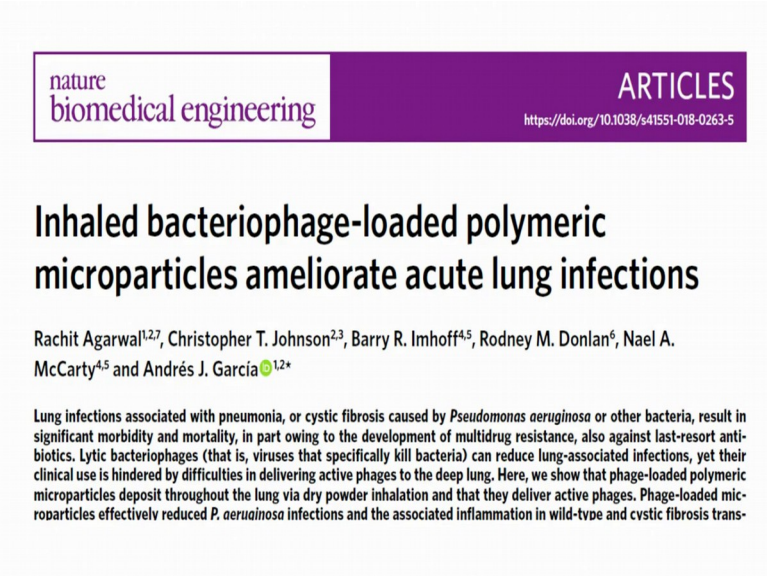

So, in the previous classes we have discussed how, if you are trying to deliver something to the lung, you can directly use inhalation based therapies to provide any side effects in the other parts of the body. It does not make sense if you want all your drug to go to the lungs to deliver it through inter venous or oral route because, that way it will distribute throughout the body. So, in this paper the authors have looked into how they can treat some infections using some polymeric particles and bacteriophages and we will describe this in a lot more detail as we go along in this paper.

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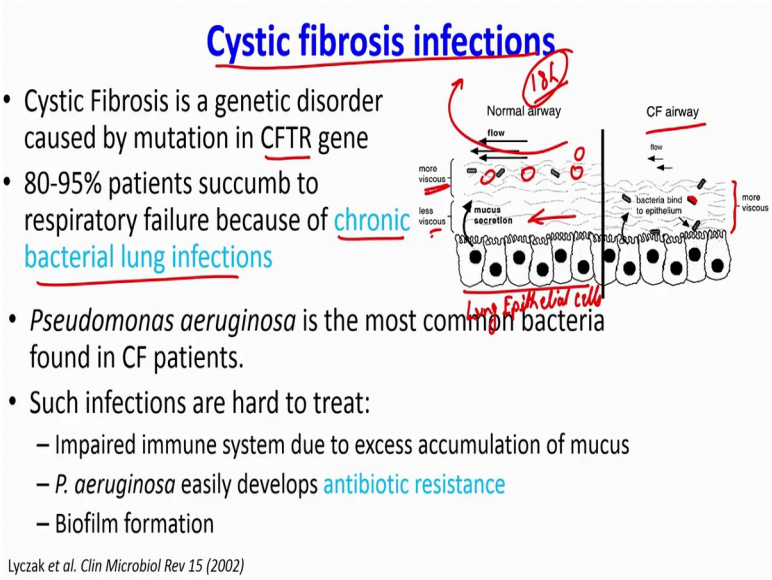

So, very quickly first of all what they are trying to do; so, this is a paper that is looking at cystic fibrosis infection. So, cystic fibrosis first of all what is that? It is a genetic disorder, caused by a mutation in a gene which is called CFTR, which is a Cystic Fibrosis Transmembrane Receptor gene. And, because of that the secretion of the chloride ion is not good and that causes a lot of problems in terms of the diffusion in the water content of the mucus. The cystic fibrosis actually affects a lot of organs including your intestine as well as lungs and others like even the sweat glands.

But, the major manifest of this comes in the lung where typically in the normal lung you have these lung epithelial cells. And, these lung epithelial cells continue to secrete lot of mucus which causes the generation of two mucus layers. So, you have a more viscous mucus layer and you have a less viscous mucus layer. And, in general in a normal airway of the lung this mucus keeps on moving up and when I say up its basically up in the sense that, it moves from lungs towards the mouth. And, what happens is let us say we are breathing any pathogens or any kind of particles that the body does not want these particles get deposited into this thick mucus layer.

And, since this mucus is constantly moving up which is called the mucociliary escalator, they will continue to clear this away. And, eventually a homeostasis is maintained where more and more mucus is secreted, whatever the previous mucus has gets to move. So, typically as it is known in the literature, it is about takes about 18 hours for the whole

mucus to get regenerated. So, within 18 hours in a normal lifespan all the mucus that is present will get replaced by a new mucus. But what happens in the cystic fibrosis airways is that, because of this problem in the gene here the water content is much lower. And, hence you have a very thick single layer of mucus.

Now, this mucus being so viscous it does not really move like it used to move, in the normal case plus it is also very thick. And so, that results in quite a bit of accumulation in blockage of the airways also. So, now you are breathing in the bacteria, which can grow into your lungs and actually remain there for quite a long time establish itself and cause the disease. So, in general about 80 to 95 percent of the patients actually come because, of that respiratory failure. Because, it is caused by this chronic bacterial like infections that will cause inflammation with more and more immune cells are going to come.

They are going to keep on destroying the surrounding tissue and that causes more mucus secretion and more homeostasis to get disturbed the airway blockage. So, eventually then the patients just die because, even they cannot breathe very well. So, as I said then, one of the major target, is this bacterial like infection and among the bacterial infection that is seen in this disease there are many types of bacteria that starts to colonize your lung. But, among them the very common sort of pathogenic bacteria in this case is Pseudomonas *aeruginosa*. So, it is a gram-negative bacterium that colonizes your lungs if you have cystic fibrosis and such infections.

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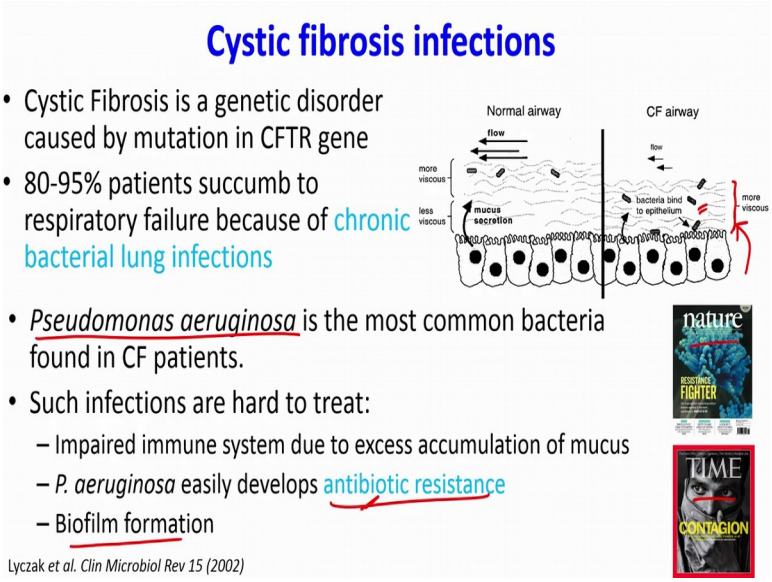

Actually, it's very hard to treat first of all and there is already an impaired immune system that is present in the surrounding. As this mucus is so thick, that the immune cells are also finding it hard to move around in this mucus. Then this Pseudomonas *aeruginosa* actually develops antibiotic resistance to whatever antibiotic you are giving, and it is a big problem these days. And, since it has a longer residence time because the mucus is not moving, it can form these biofilms.

And, biofilms in general are extremely difficult to treat, we have talked about this in our infection class. These biofilms will be very difficult to treat as the antibiotics do not really work very well, you almost eat 1000 times antibiotic concentration which could be extremely toxic to the healthy cells also and it is very difficult to treat. And, in general I mean; obviously, the antibiotic resistance is being highlighted quite a lot in several journals and several publication magazines. It is a big problem that is being faced by humanity right now.

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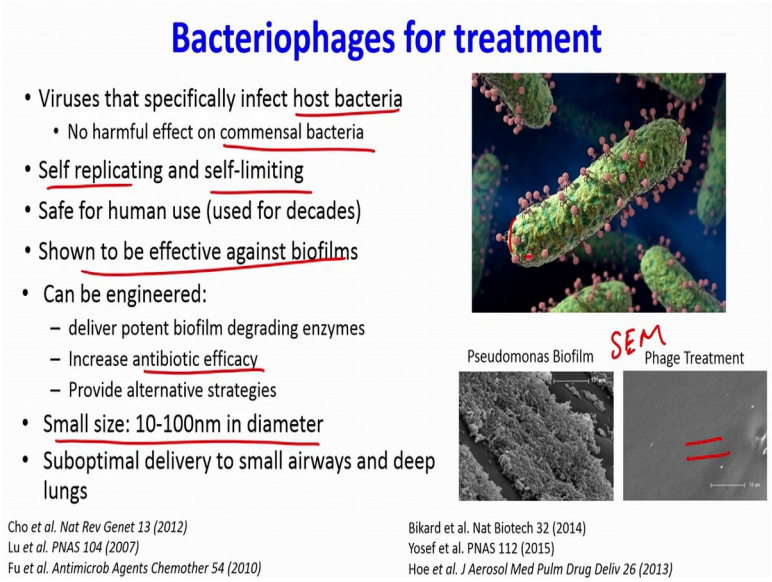

So, this paper tried to tackle that and what they have done is that, they are proposing, again they are not the first one to do that, there are several people who have proposed that. But, in this case they are looking at a normal delivery method for this solution which is bacteriophages for treatment. So, what are bacteriophages? Bacteriophages are nothing, but these are viruses that specifically infect their host bacteria. This is just a model image; you can see that there is a bacteria and then several types viruses are attached on to on to the top of this bacteria.

And so, these are viruses that are going to go and in put their genetic material in the bacteria eventually causing the lysis of this particular bacteria. These bacteriophages are very specific for their host bacteria. So, they do not really harm your commensal bacteria, so we know that we have a lot of bacteria in our body that is actually healthy and our body requires it. So, these bacteriophages are not going to kill it, unlike your antibiotics, which wipe out nearly all of your bacteria at a time; these bacteriophages are specific for the target. And, they are also self replicating and self limiting and what exactly does that mean is that, when they find the host they will start to multiply. So, they will self replicate and when the host is not there they do not multiply anymore.

So, they limit themselves and get eventually cleared out, they are safe for human use they have been used for decades in humans in fact, the they have been used even before the antibiotics were discovered. So, there is a long history of use in humans and so, they

have been shown to be safe and they have been actually shown to be effective against biofilms as well which is not the case with antibiotics. So, this is just image of pseudomonas biofilm; this is a SEM image: Scanning Electron Microscope image. And, it shows that the surface is colonized by the Pseudomonas biofilm which when treated with the phages gets completely eradicated. Other thing is, like any other viruses or any other genetic material they can be engineered.

So, you can put other enzymes into these bacteriophages also to increase their potency, they can increase even antibiotic efficacy. So, you can use by co-delivery with antibiotics or you can have you some alternative strategies also; however, one challenge is the size is fairly small. So, they are about 10 to 100 nanometers in diameter and which if we are looking at dry powder delivery to the patient, which is much more compliant. They do not really go to the deep lungs and we have talked about this in our inhalation class, we will go over it briefly again.

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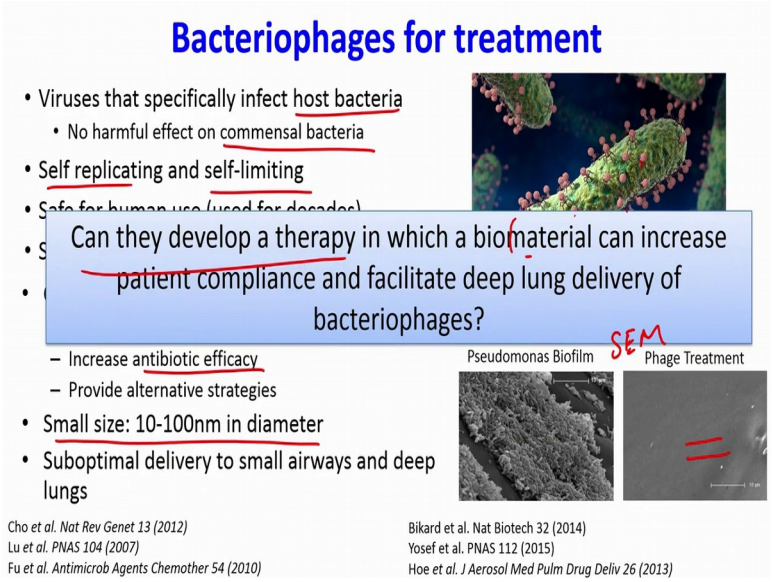

So, the whole point of this paper was can they develop a therapy which uses a biomaterial; so, that they can increase the patient compliance as well as ensure deep lung delivery of these bacteriophages.

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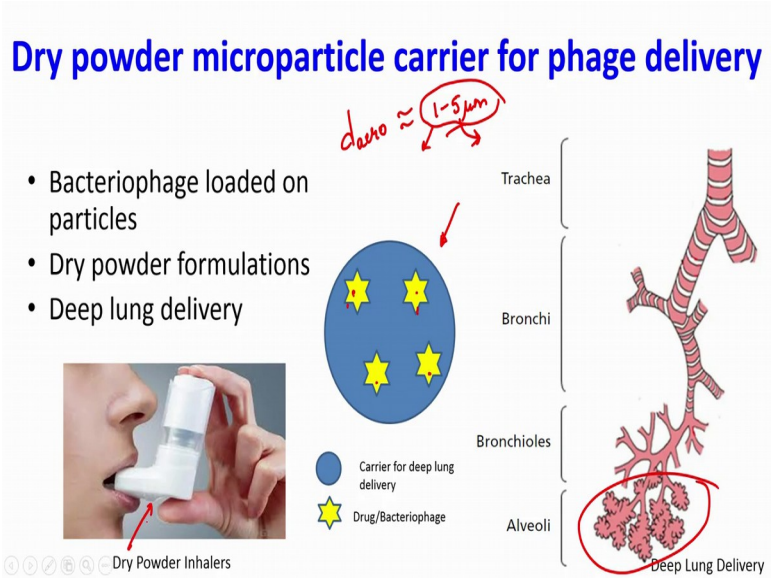

So, again if you remember one of the things that causes delivery in the deep lung is the aerodynamic diameter. So, that d aero and we said that the d aero has to be between 1 to 5 microns. And, that is because if it is lower than 1 micron then typically, they do not have enough inertia and then they keep on going in streamlines with the air that you are breathing in and they also get exhaled out. If they are larger than 5 microns then they tend to have a very high inertia and they end up accumulating where, they first hit which is that trachea in the upper mouth, upper airways. And so, the ideal range you want to deliver them, to is 1 to 5 microns.

So, this is what the system was envisioned by these authors where they are saying that we will use a particle which is in this range. And, then load these bacteriophages or the drug whatever you are trying to deliver onto these particles and that way this will ensure that these particles go deep in the lung, in the alveolar sacs. And, then there is when the drug can release out and obviously, they want to use the dry powder inhalers because it is much more patient compliant, the patient can just use it by themselves.

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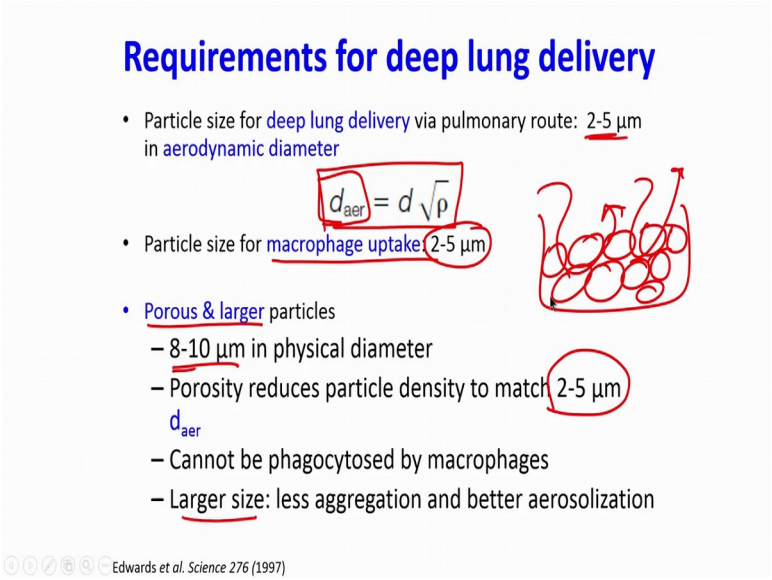

So, again what are the requirements? As I said we have to have 1 to 5 microns for the deep lung and the d aero for polymeric particle we had also discussed before. It is nothing, but its related to the physical diameter and the square root of the density. So, now, we also know and we have discussed this; also that the macrophage have very high uptake in the range of 2 to 5 microns. So, if you deliver something in that range what will happen is most of your drug will end up in the macrophages: alveolar macrophages, that are surveying the area and you will lose a lot of your drugs.

So, that is not the outcome you want; you do not really want to deliver this drug to the macrophage because this bacterium does not reside in the macrophages. So, one thing that they came up with is, they decided to make these particles porous and larger. So, they actually changed the physical size of these particles to 8 to 10 micron and they decrease the porosity of this to keep them at 2 to 5 microns, range for that d aerodynamic diameter. So, at that size range of 8 to 10 micron they are not too big.

They are no longer than this range of 2 to 5 microns which the macrophages can take, but they are still in the right aerodynamic range to be able to deposit in the deep lung. And, then another benefit that they observe from this is the larger the size of the particles the easier it is for it to aerosolize. So, if you have a powder and you flow, let us say if you have this large particle powder. So, the surface to volume ratio is low.

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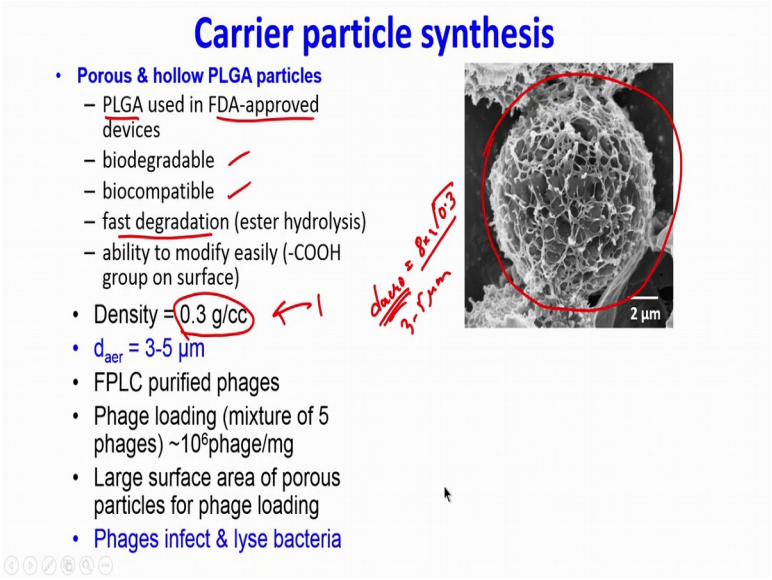

And so, the air can come in very easily and aerosolize it and so, that is what they went ahead and did they used the polymer called PLGA; again, we have discussed quite a bit through this course which is an FDA approved polymer. And, they have been able to make this porous particle, as you can see its very highly porous.

So, its density is very low, it is biodegradable, it is biocompatible because PLGA you can have it faster degrading and the density that they modified was about 0.3 gram per cc. So, from 1 they have dropped down to 0.3; that means, that now this particle is fairly light and now if you apply that equation of daero, let us say if this is 8 micron multiplied by the square root of the density.

So, now if you solve this you will find that this is more in the range of 3 to 5 micron. So, that is what they used here; then what they have done is they have used bacteriophages. They found bacteriophages, which specifically targeted against pseudomonas and they have purified using a procedure called fast performance liquid chromatography. And, eventually they have gone ahead and loaded these phages onto these particles and what they found was that they get a loading of about million phages per milligram of particle and the loading is done by simple adsorption.

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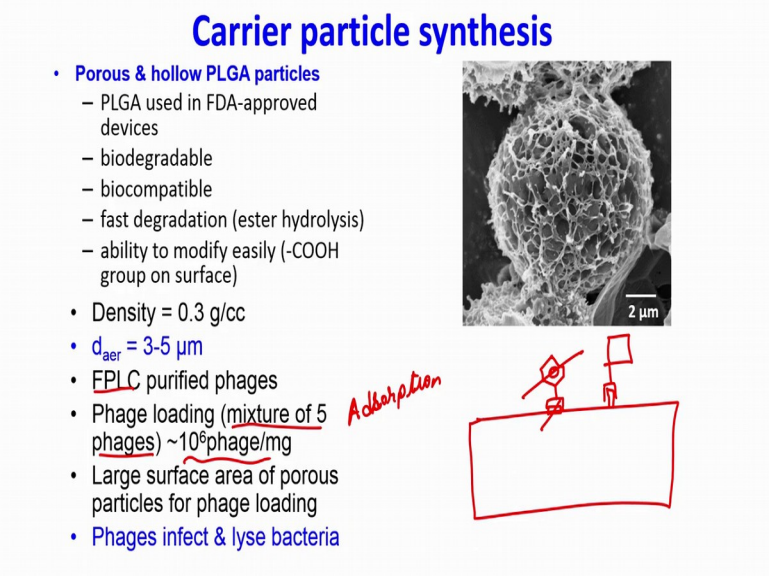

So, the phages are just incubated with this particle and they have let them interact with each other and adsorb onto the surface. And, then they find that they can put about 10 to the power 6 phage per milligram of particle. It is important to note in this case they are using a mixture of 5 phages and the reason they want to use mixture of phages is because let us say this is the bacteria and let us say this is a phage. So, maybe there is a receptor on the bacterial surface to fit where the phage goes and binds.

So, if they only use one type of phage what will happen is the bacteria can potentially mutate this receptor to which then the phage cannot bind. So, to prevent that from happening they have used 5 phages at a time and so, this is phage 1 maybe the other phage uses a different receptor. So, maybe there is another receptor now, which is being used by a different phage. And so that means, that even if let us say a bacterium is able to somehow nullify one of the phages, it will still be susceptible to the other four phages. And, that ensures that the therapy successful and then they were able to show that these phages that are loaded onto the particles are still active and goes ahead and lysis the bacteria.

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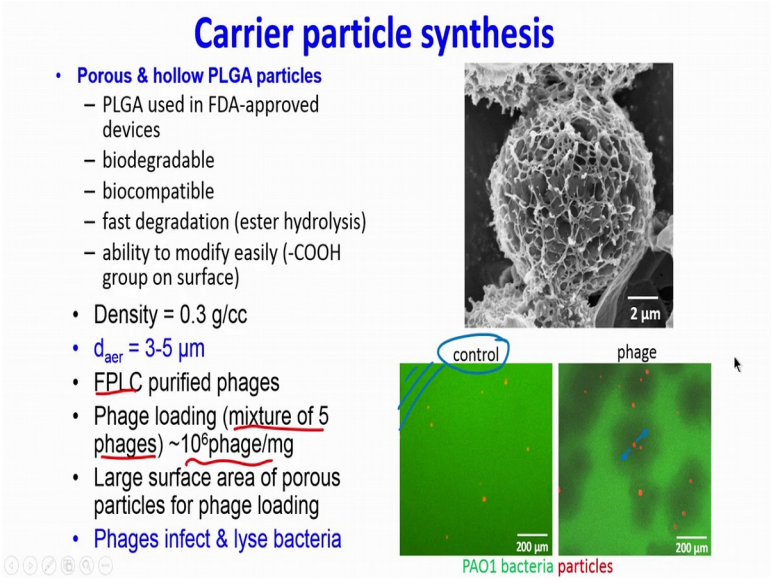

So, here is what they have shown. So, they have used a GFP bacteria, and in the control; so, has all of this is a bacterial lawn and the red is their particles. So, wherever the particles are landing you do not see any bacterial death. However, if they load their particles with the phage you see not only is the green bacteria getting cleared wherever the particle is landing and is actually propagating. So, as I said the phages are self replicating. So, they are infecting the bacteria, they are lysing it, they are spreading and then they are also killing the bacteria next to them as well.

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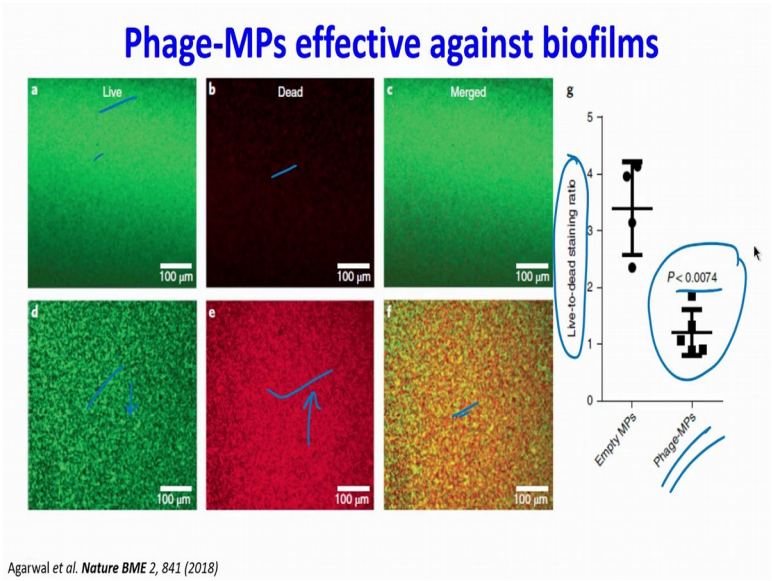

Then here, they have shown that these phages are also effective against the biofilms. So, now what you are looking at, is a biofilm which is being stained by live dead. So, you can see that in this case there are quite a lot of the biofilm that is alive versus the dead. Whereas, if they give their phage particles, they see quite a low intensity of live bacteria and quite a high intensity of the dead bacteria and here is the merged image; this is for the quantification of live to dead in the tissue.

And, in cases where they are giving the phage combined with micro particles, they find that the ratio is much lower actually significantly lower. So, they are effective even against the biofilms.

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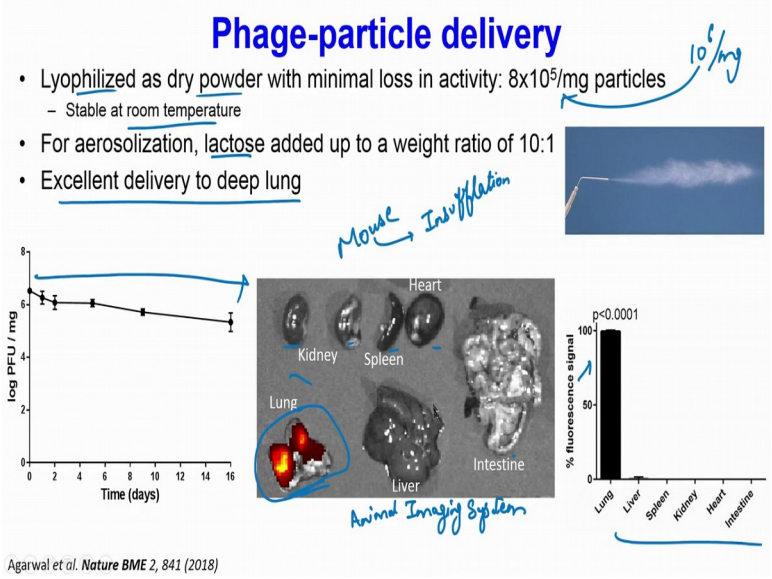

Then they have went ahead and made the dry powder formulations. So, what they have done is they have lyophilized their powder or they have lyophilized their particles to make a powder and they find that there is a minimal loss of the activity. So, I told you that they have been able to get 10 to the power 6 phages per milligram and you find that there is not a whole lot of loss and this powder is fairly stable at room temperature. So, here, you can see that here is the titer and it does not really drop much over a period of 2 weeks at room temperature also. So, this could be a good therapy for even village locations which may not have enough access to let us say a cold storage or any such facilities.

And, to further help with the aerosolization, they have used lactose which is a cryoprotectant as well as a good aerosolizing agent. So, it is essentially nothing, but a sugar and so, they have mixed this along with the particle formulation so that it starts to flow better. PLGA itself does not flow very well. So, they have to mix it with lactose to do that and in their result, you will see that they find that this is excellent delivery to deep lung. So, here is what they have done; here they have done it on a mouse and then they have isolated various organs.

So, this process is called insufflation where the mouse trachea is accessed and then through a syringe, they are puffing the powder directly into the trachea; so, that process is insufflation. So, as expected you see and these particles are fluorescently labeled and then they are using some kind of imaging system animal imaging system. And what they find is, they specifically get delivery in the lungs whereas, the other organs do not show any end delivery at all which is what you expect if you are doing in simulation. This is further quantification that all the fluorescence signal is from the lung itself and all the other organs do not show anything.

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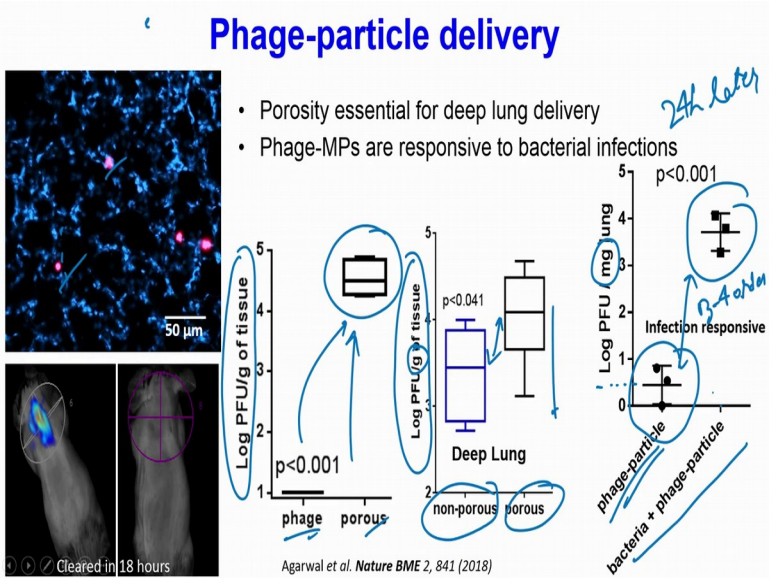

Then they have went ahead and euthanized some of these animals and then did the cryosectioning and so, what you are seeing here is cryo-sectioned lung. This blue is the DAPI which is staining the nucleus of mammalian cells and red is your particles and you

can see that even in the deep lung. So, you know this is deep lung because, you can see quite a lot of air gap which suggests that this are alveoli in the histology.

And, you can find that these particles are depositing in the deep lungs and hence they are good in terms of their aerodynamic diameter range and this is the further quantification on live animals. So, they have done the whole animal live imaging and what they find is most of the signal is from the lung whereas, in a healthy animal, if you wait for about 18 hours it gets cleared away.

So, its generally safe for a healthy animal it does not really stay in the lung for long, it does not block anything. It gets cleared away within the 18 hours, which is what is expected as I said in healthy animal the mucus is going to regenerate in about 18 hours and then they show that the porosity is actually essential. So, if they make the same size particles which are non-porous and then they insufflate those particles into the mouse lungs. What they find is, first of all if they compared with a free phage, they find that the porous particles are able to give a very high titer of phage to the lungs.

So, the experiment is done that you have insufflated either free phage or phage loaded on the porous particle equal amount. And, then you homogenize the lung and titer for how much phage is present in those homogenates. And, you find that almost 4 orders of magnitude, mainly because, higher the particles and the same thing they observed with the non-porous versus porous particle. So, the porosity is essential for deep lungs and what they find is in the deep lung region the porous particles give you about an order of magnitude higher deposition of the phages compared to non-porous particle; again, remember this is titering for the plaques. So, all of these phages are actually active phages.

So, all of throughout this process they have been able to attain activity of phage in the lung and then finally, they show that this is actually infection responsive. So, and what that means, that let us say if it is a healthy lung then you find that if you deliver phage particles you get a certain titer of your phages in the lung remember and this is in milligram now, so that is why you see a drop in the scale and this was in microgram, this was in gram. So, but in any case, if you use these phage particles, they give a certain accumulation in the lung. However, if you have infected lung these phages then replicate so, this is done 24 hours later.

So, these phages replicate and actually increase their number by again almost 3 to 4 orders of magnitude; indicating that these are actually self-replicating and infection responsive system.

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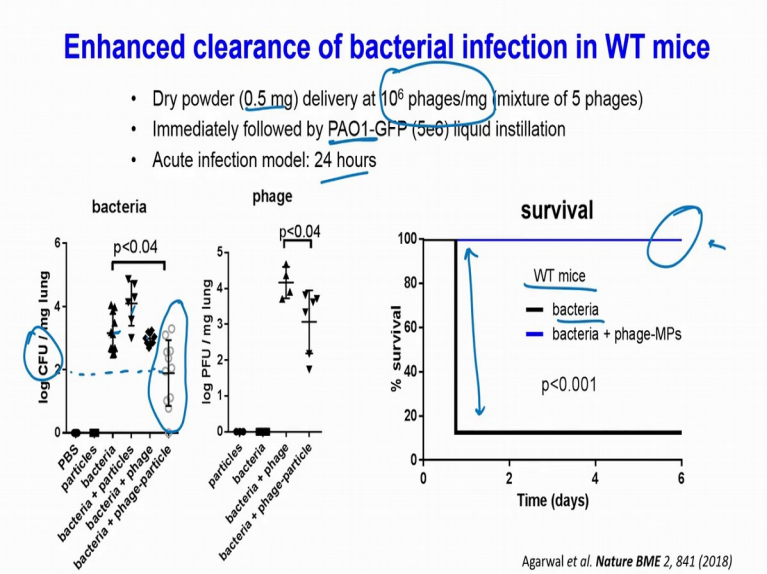

Then they went ahead and used this system on an acute infection model. So, what they did is they delivered the bacteria in the phages to the mouse in the lungs. And so, these are healthy animals, in this case wild type animals and they have delivered about 0.5 milligram of particle. It has a loading of the phages in that and PAO1 which is a lab strain of pseudomonas was used and this is a 24-hour model. So, both of these things have given and then they are testing for what happens 24 hour later in terms of both the bacteria in the phage.

And, what they find is only in the case when they have the phage loaded on the particles. So, here is your free phage, here is your only bacteria, here is your only particle. So, you do not see any reduction, but only in the case when you have particle loaded with the phage you see a reduction in the amount of CFU, some number of bacteria that is present in the lung homogenate. And, then the phages are also measured and the bacteria was then the animal was then looked for survival and what they find is you get a massive reduction in the survival between the two groups.

So, if you do not give any treatment most of these animals die; only maybe 10 - 15 percent of them survive. But, if you are giving phage treatment almost in fact, 100 percent of the animals ended up surviving for the duration of the study.

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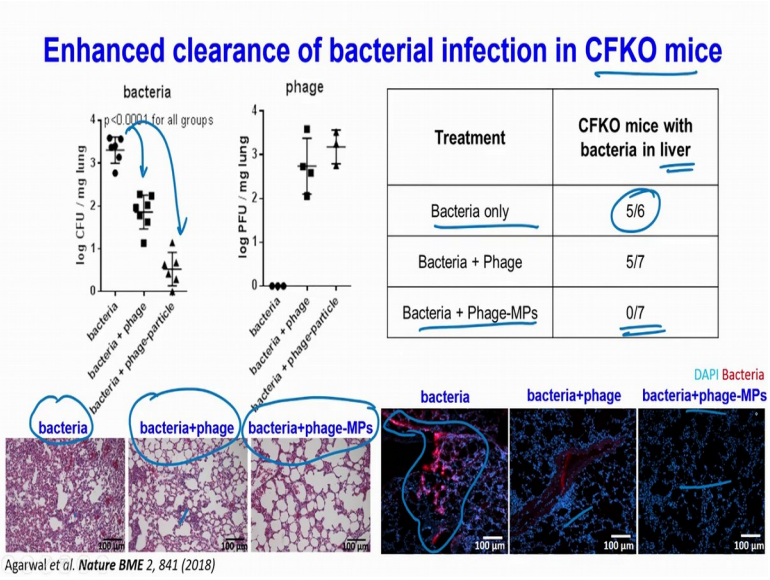

And, then they use a CFKO mice which is a mouse that is mutated and mimics the cystic fibrosis. Here also they are able to show that in fact, at a much better rate that if you use only phages there is some effect. But if you use phages plus bacteria you almost eradicate all the bacteria that is present in the system and here, they are also showing whether the bacteria is spreading or not. So, they have analyzed for liver where if the bacteria it does spread systemically, it will accumulate in the liver also. And, they find that if they give the bacteria only treatment almost all the animals have bacterial colonies in the liver. However, if they use a phage microparticles none of the bacteria is found in the liver.

So, not only is preventing the lung, it is also preventing other organs as well and this is more histology data. So, in an infected animal you see if you only give bacteria you see a massive response of your immune cells. You can see where alveoli, which are now completely filled with all these immune cells you can see here. If you give some phage treatment then this reduces a bit, but it almost becomes like a healthy tissue; if you do it with the phage micro particle. And, this is again further showing the number of bacteria

that is present in these lung sections. So, you can see all these reds that is being represented as bacteria and that reduces further as the treatment is given.

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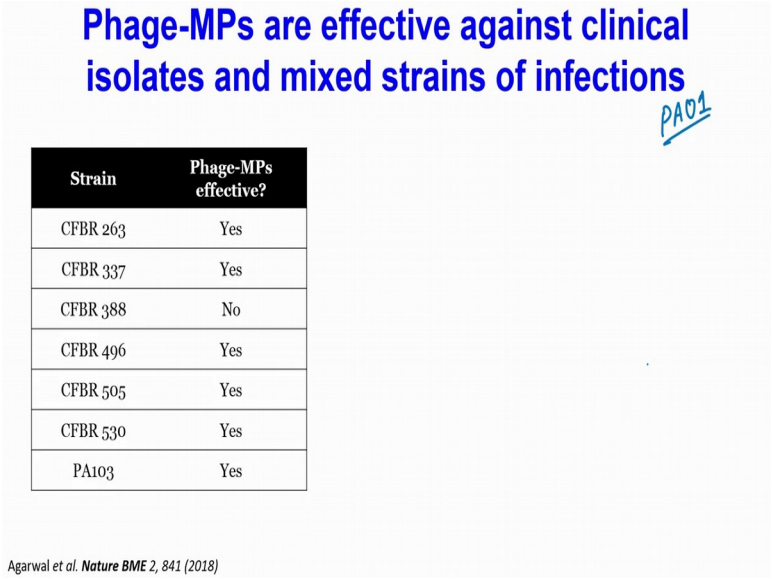

Then they wanted to see whether something like this is going to work on clinical strains. So, I said the authors initially used the lab strain that was PAO1. So, lab strains are good to do your optimization, but obviously, the clinical strains are much more virulent and much harder to treat. So, what they did is they have checked it on various strains of bacteria that are isolated from the patient. And, what they find is their phage microparticles is able to kill most of these; there are one order here, that it was not able to kill. But, nearly all of these clinical isolates were being able to be killed by these phages' micro particle formulation.

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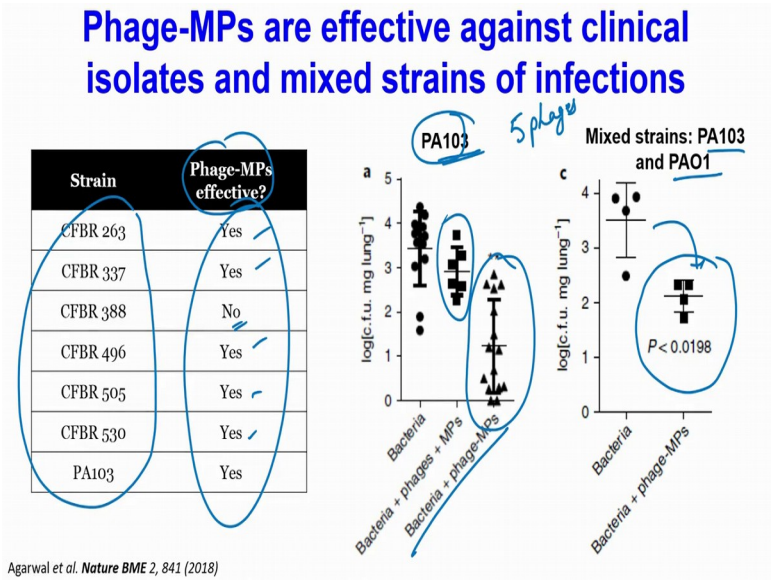

And then, they did an in vivo experiment using one of this clinical strain, which is PA103 and what they find is again the same result that, if they only give phages, they do not really see much reduction. In fact, there is no significant reduction whereas, if they give their phage micro particle, they see quite a bit reduction in the number of bacteria that is present in the lungs. The other thing they did is obviously this works well with a single infection bacterium in mouse system. But humans are exposed to several types of bacteria, several types of Pseudomonas, several strains of Pseudomonas at a time.

At a time, patient can have many types of Pseudomonas strains that are colonizing the lungs. So, how can this work with all that? Because they are using the 5 phages and then they have shown that these phages are effective against most of the strains that are found in humans. They went ahead and tried that out and they find that, even if you have two different strains mixed together and then you give the phage therapy even then it can cause the reduction in the number of bacteria that is present.

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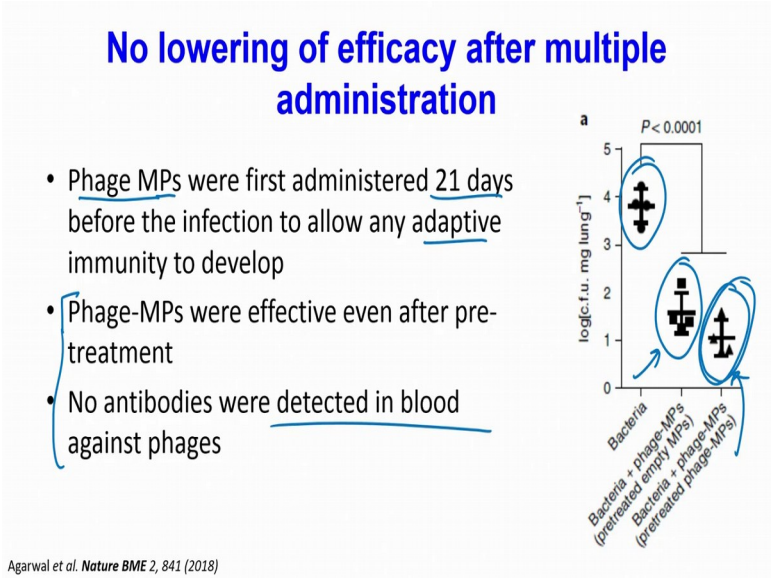

And then one question that repeatedly arises with the phage treatment is what about the immune response against the phage itself; so, because phages are foreign and they can elicit some immune response. So, in this case then the authors wanted to test what happens if they give phages before and then try to therapy. So, in this particular experiment they first used the mouse to deliver the phage microparticle without any bacteria. They waited about 21 days which is a good enough time to let the adaptive immune response to develop.

And, then what they did is, then they went ahead with their originals with their normal study where they use the same mice to then, give both the phages in the bacteria and saw the response 24 hours later. And so, what they find is if you have pre-treated versus no pre-treatment of phage. So, in this case there was pre-treatment with phage, in this case pre-treatment is only with particles and obviously, this is which was never exposed to phages. What do you find is both of them works as good and there is no difference between the therapies?

So, if there was immune response against phages the body would have tried to clear the phages and you would not have got this much clearance. And so, at least in terms of their functional efficacy they do not see any reduction. They also looked for antibodies against phages in the blood and they could not find any antibodies and use the phages at least for one administration. Obviously, this needs to be done at a much longer duration and in a

chronic model to be able to successfully say that this is going to work or not. But there is a lot of promise in this particular therapy. Okay! So, we will stop here and we will continue in the next class.

Thank you.