Drug Delivery Principles and Engineering
Prof. Rachit Agarwal
Department of BioSystems Science and Engineering
Indian Institute of Science, Bengaluru

Lecture – 63
Nanotoxicology and Translation Pathways

Hello everyone, welcome to another lecture for Drug Delivering Engineering Principles, and this is going to be a last lecture for this course we have covered quite a lot of this course and you will see today we talk about toxicology as well as regulatory pathways. And I hope you have learned quite a lot from this course, so let us finish what I have to talk about today.

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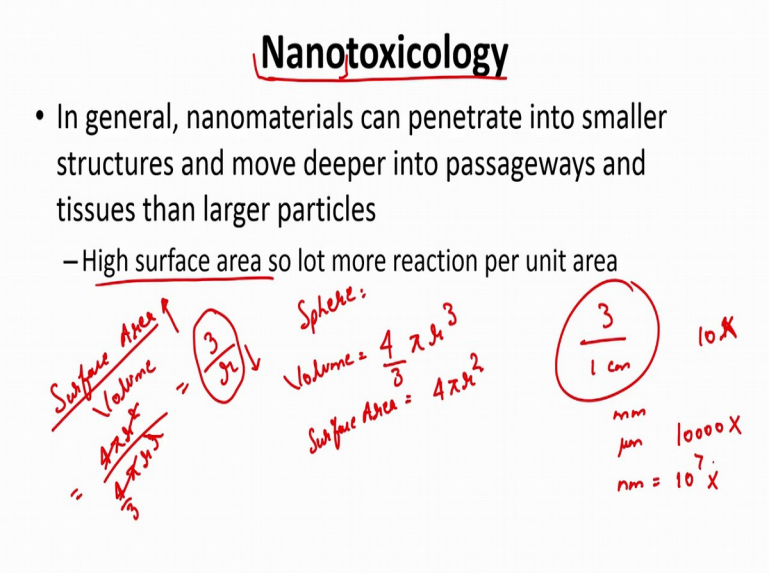

So, again we have talked about so much of particles micro and nano particles all of this has been shown to give quite a lot of advantages in terms of drug delivery whether it is sustained release, whether it is triggered release, whether it is preventing any surgeries and going to different parts of the organ or maybe some other application giving intracellular going to niches where the drug may not itself go.

So, but then when you make these particles of these size ranges they again attain some other properties which may not be there at a macro range and then some toxic effects may be seen. So, that is where the toxicology comes in, nanotechnology as the name

would automatically say, this is dealing with how the nano part of it or the nano property of your device is causing toxicity to the body. So, how can one go about doing this.

So, in general since these are nanomaterials these can penetrate into a smaller structures much more easily ,they can move much deeper into the passageways and tissues then larger particles. So, again if just to give you a very simple example let us say I have this pen this cannot penetrate through my skin it is too big unless of course, it is been given a lot of momentum and it damages the tissue. It cannot go through my skin, but when we talk about these particles it is such a minute range my skins my skin may itself be porous and some of these can go through.

We have talked about transdermal delivery; we have talked about various kinds of delivery. So, it is then fairly feasible for some of these particles to be able to penetrate through the skin. So, again some of the reasons why this can happen is first of all they have a very high surface area and so they have a lot more reaction site per unit, hence they are very reactive. So, what do I mean here is a let us say, so the volume is what for, but let us say we take about sphere.

$V= 4\pi r^3/3$

Now, let us say we are talking about, so this is basically the volume, then we have the surface area which is nothing,

Surface Area = $4\pi r^2$

Now, if I am trying to compute for a given volume how much surface area is available. So, what I can do, is I can just simply do the surface area by the volume. So, if I do that will basically give me

$4\pi r^2 /(4\pi r^3/3)$

and that is going to give me

Surface Area/Volume = $3/r$

So, what I am saying that as I am reducing the r, since r is in the denominator my surface area to volume ratio increases. So; obviously, this r for a macro object for let us say a soccer ball or something else and this is in centimeters or millimetres, so at that point let

us say it is in centimeters. So, we are talking about 3 by let us say 1 centimetre, so you get a certain ratio. But now I am some decreasing this it became millimeter this ratio goes up by 10 times, so it becomes 10 x.

Similarly, if I decrease this down to a micron range. Now, I am talking about another 1000 times so, 10000 times, I have increased the surface area for a given amount for the given volume. And then similarly if I go to now nanometer range I am talking about this increment of 10 to the power 7. So, 4 and 3 here, 10 to the power 7 times I have increasing surface to volume ratio.

So, this is what we will continue to do here in the field and we have also talked about it in the earlier classes that as you become more and more small your surface to volume ratio increases. So, now what this means is a nano particle compared to a centimeter size particle will have almost 10 to the power 7 which is 10 million times the surface area.

The way these material will interact with the various reagents, various things in the surrounding is through the surface area. So, now, you have a lot more reactive sites which were earlier buried in the bulk volume, but they are now available on the surface. So, again this is a good thing to have for that is why the nanoparticles are being used quite a lot, but then it comes with shaded side effects where they can also have some toxicity induced through them.

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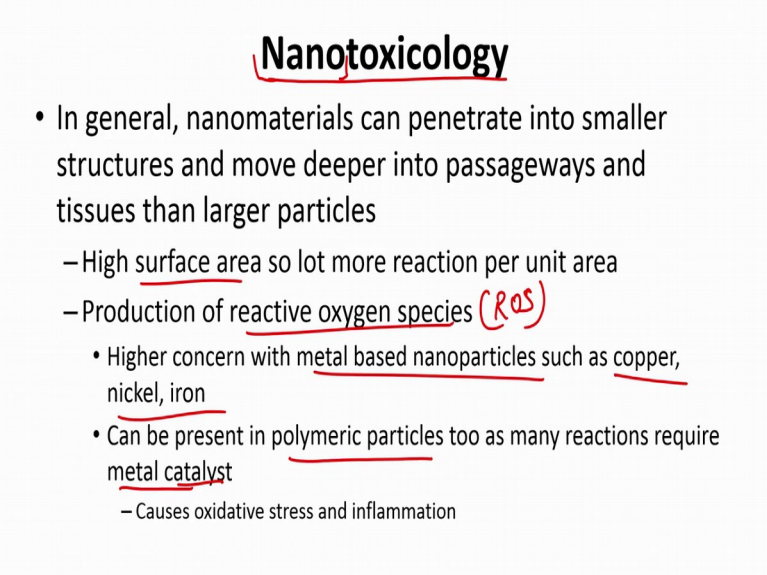

So, they can also produce reactive oxygen species, the this is much higher concerned with metal based nanoparticles rather than the micro particles or the polymeric particles, because metals are a good oxidizing agent some of them good reducing agent. So, all of this results in such as copper, nickel, iron they can create reactive oxygen species.

Now again you have lot more surface area, so then amount of reactive oxygen species in the being generated is quite high and these reactive oxygen species are also referred to as ROS are a part of the inflammatory process. So, now, you are increasing the inflammation, if the inflammation is too much you may even have quite a lot of toxicity fever and things like that will come up.

And this could be also present in polymeric particles too because lot of these polymers are required to have metals as catalysts. So, some of these we talked about PLGA requires some catalyst from a synthesis from the lactide and the glycolide. So, these metal could be present in some trace amount and they can again be causing this oxidative stress and inflammation.

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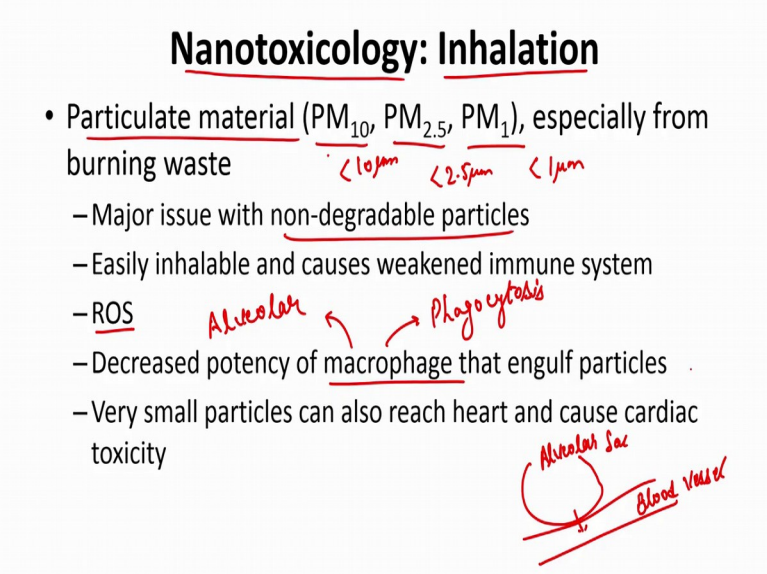

So, this is just one way, then we can break down this toxicology into various forms. So, let us start with the inhalation since this is by far one of the most sort of a topic that is currently in a lot of debate with the air pollution in all these materials that are out there including some exhaust from the vehicles and all. So, any of us have heard this particulate matter mid particulate material PM 10 PM 2.5 PM 1 and especially from

burning waste. So, what are these PM 10 is nothing but a particle which is less than 10 micron.

So, any particle which is less than 10 micron is classified as PM 10, PM 2.5 is something that is less than 2.5 micron and PM 1 is something that is less than 1 micron. So, you will often hear about people saying that the air quality is bad the PM 2.5 is very high, the PM 1 is very high, the PM 10 is very high. That basically means that the air is carrying lots of these particles and flowing them around and we are breathing them in we are inhaling them it is going and depositing in our lungs.

Some of these material become quite high number if you have burning waste or if there is a building construction that is going on and a lot of a dust particles flying around. So, typically this is a major issue with non degradable particles. Now, in cases of therapy we make lots of these and people are now inhaling this something like a normal inhaler which people use in asthma. They are also exposing this to the environment and then this may increase in number as more and more users comes in for these nano micro particle.

So, there is some toxicity associated with that is especially for non degradable particles, because degradable particles will go away ,will degrade either in the environment or in our own body, but then this non degradable particle will remain. So, these are easily inhalable and cause , weakened immune system, again like the metal particles these can also result in the reactive oxygen species. As I said these macrophages they represent in the lung they are the ones that are responsible for phagocytosis and in this case we are mainly talking about the alveolar macrophages.

So, these alveolar macrophages will try to engulf anything that is depositing in the lung and there is several of them, but then eventually if the load is very high of these PM 10 PM 2.5 PM 1. They may reach a saturation level where they cannot engulf anymore, they cannot be graded, it is non degradable particle. So, where do these particles go is macrophages will either become non functional or they will actually even become aberrant and they start secreting lots of things for inflation for inflammation to kick in.

And then some if the particles is actually very small then we know that these alveolar, let us say if this is an alveolar sac and this is a very thin membrane between a blood vessel and these alveolar. So, if they are extremely tiny particles they can actually go in through

this barrier and they may even reach heart and brain. So, even through inhalation you are causing it to distribute systemically.

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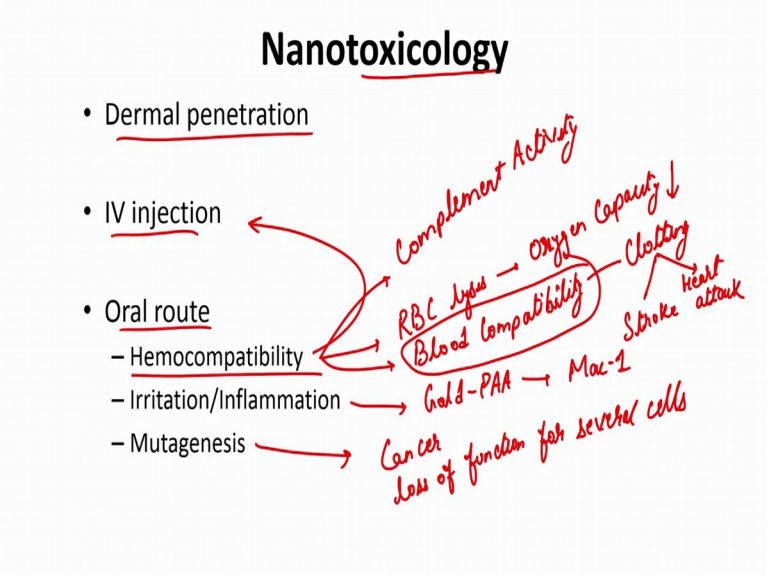

So, and then there are some other routes as well for another toxicology and the one is dermal penetration which I briefly mentioned that things can go through your skin, there is IV injection. So; obviously, if you are designing these particles to be injected IV they may still start to accumulate in the body and then of course, to the oral route if it is in a tablet form or in any other form. T he other things we should take care for all of these is the hemo compatibility.

So, if it touches the blood whether it is actually or fertilizing the blood vessels and the RBCs, so RBC lysis a big concern. So, if we have a lot of RBC lysis is happening because these RBCs are interacting with these material then your blood count is decreasing you may not have enough oxygen carrying capacity. So, oxygen capacity will go down, the last thing you want is these particles may induce let us say blood clotting, so this is a separate thing.

So, something which we had discussed quite extensively in our inflammation part of this topic, you know blood compatibility in which is the same thing, but essentially we are talking here about clotting. So, if it induces clotting it may cause things like stroke and heart attack because these can go in and clog the blood vessels which are feeding either to your heart or to your brain.

Then similarly this can result in complement activation which again is going to lead to inflammation, we discussed other ways as well right. We discussed that these one of the papers we had discussed which was the gold PAA particles that were causing Mac 1 receptor activation.

So, some of those processes may also be involved, it does not have to be directly complement or the clotting itself it may be is through some other way. And then finally, if the cells are taking it up and there are some mutagens and they may even cause cancer or loss of function for several cells.

So, all of these are some of the things that we need to keep in mind while we are designing nanotoxicology and that is why it becomes very important and we had gone over blood compatibility, immune system and complements all of this is again very very important. Because eventually most of these drugs will be given to patients who are somewhat sick, they may not be as functionally active to fight off any challenge as let us say a healthy person. And the last thing you want in the in case is to then make the disease worse rather than letting it go in that normal scenario.

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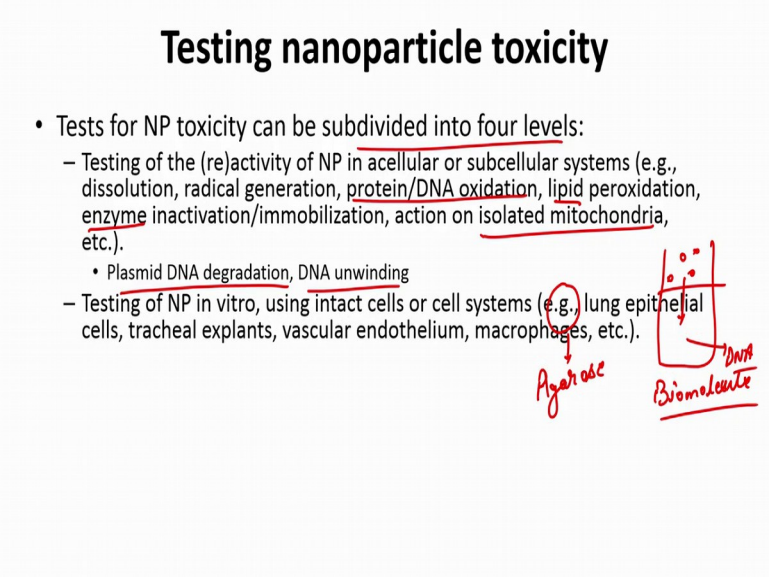

So, how would you then go about testing this. So, again some of those things we have talked about already throughout this course that whether it is compatible, whether you can change the property, copolymerization, you can conjugate peg to make it more

stealth. Some of these things we have discussed and then in the more systematic way we look at here.

So, basically the testing of nanoparticle toxicity can be divided into four levels. So, one is to testing the activity of the nanoparticle in cellular and subcellular system. So, for example, whether it causes radical generation whether it causes oxidation of a biomolecules, protein and DNA, lipid peroxidation if there is a important enzyme whether it is affects the enzyme, this is again just in a nature in the environment.

So, you have a tube, you have let us say DNA or any other bio molecule in here. You add your particle suspension here and see if the function of this DNA or the bio molecule or whatever it is, whether it is enzyme or any structural changes are observed. You can even isolate mitochondria and test it quickly to see if the mitochondrial function is being disrupted. So, these are some of the acellular systems that you can check.

So again as I said you can look for plasmid DNA degradation. So, let us say if I have a plasmid and I can run it through a page gel or an agarose gel and if I see that the band has shifted or it is slightly moved up or down I can say that there is some process that is happening, the DNA unwinding you can look for by melting temperatures. The second level that you will look at is more at a in vitro level.

So, this is looking at intact cells or the cell systems. So, you can you have lots of cell lines, you have macrophage cell lines, endothelial cell lines, epithelial cell lines, you can take these cell lines and test your particles to them see whether the cells are dying.

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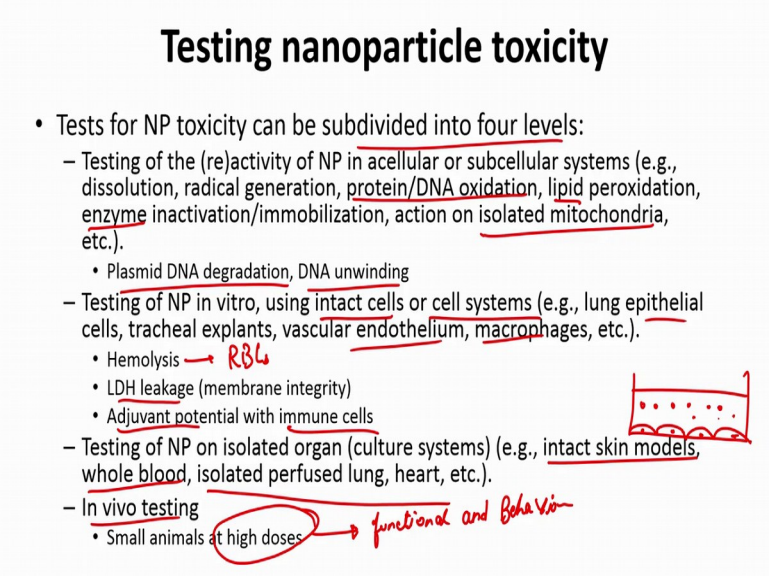

So, maybe in a dish you are culturing these cells and you can put your particle solution over these cells, you can culture them for different time points. Let us say 24 hour, 48 hours and then look if the cells are alive or dead and if they are alive then how functional they are. So, maybe look for some of the metabolic activity, some of these kids are readily available in the market that you can used to test this out.

The same thing you can do with the RBCs, so hemolysis. So, whether the blood is getting lysed with this; you can look for some enzyme leakage and to see whether the membrane is disrupted in any way or you can look at immune cells to see if the immune cells are getting activated with this. So, some of these polymers can act as adjuvants, so you want to make sure, if you do not want the immune get active you can then test these particles on your immune cells and see if they are actually becoming active then you cannot use that for that application.

The other thing is testing the nanoparticle on an isolated organ, so you can have some primary organs isolated. So, blood is a good example this is one of the organ that is very widely used and then you can also use some intact skin models, there is lots of modelling the 3D modelling of tissues have started. So, you can take these heart out, primary heart out, the cells have been alive for some time you can test your particles on those culture and see if any changes you are seeing compared to the control.

And finally; obviously, the gold standard in this case is an *in vivo* testing. So, you use some kind of a small animal you give these particles at the high doses and see if there is any functional or behavioural changes that you observe. Functional and behaviour changes that you are observing in the such animals, so this is in a nutshell how you can divide the nanoparticle toxicity.

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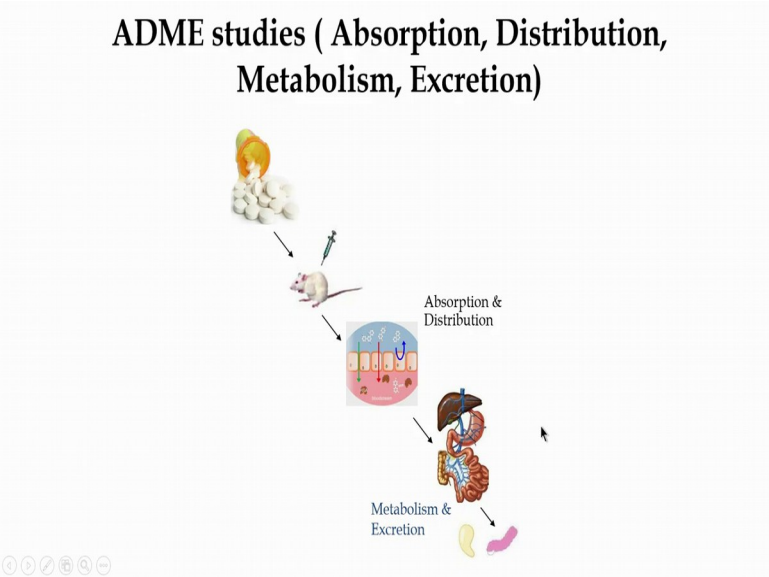

A very fancy term to this is ADME. This is abbreviated form which is Absorption, Distribution, Metabolism and Excretion. So, any molecule, any material that you are using you would like to do these studies to see how it is getting first absorbed. Once you have injected, how it is then distributing in the system, how it is getting metabolized in the system and finally, how it is coming out of the system. And which especially for non degradable particle is a big concern because if they are above 6-10 nanometer, they cannot come out, so they will accumulate ok.

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So, this was mainly for the toxicity, but what happens let us say I have a system which I think is much better than what is currently used in clinics. And I have tested all these toxicity and I found that this is good and now I want to move further and use it in patients.

So, how do I go about it, how do I get it to the patient, how do we get approvals you could do with the patient, so that is what final thing we going to talk about. So, the first step is of course, to make sure absolutely, that the product that you are wishing to market is actually feasible. So, you want to ensure that there is enough market to it, because it is a very long and winding road as we will discuss in the next few slides. And you want to ensure that the product after all that is going to be used and is actually going to benefit as well as there is a market to it.

The step two is then to determine what kind of regulatory agencies such as ICMR, how are they going to classify it. So, whether it is going to be classified as something that is eaten whether, this kind of like classified as a medical device. So, all of these will have a different sort of safety and effectiveness standard, it is going to be whether just use externally to the patients or whether this is going to go inside the patient, all of these will present different regulatory challenges.

And then the step three is the development of the data and information that you would need to submit it for a marketing application. To get clearances there are certain forms

and certain processes that you need to go through and then you will have to go ahead and accumulate all that and then submit it to the regulatory agency and get the approval.

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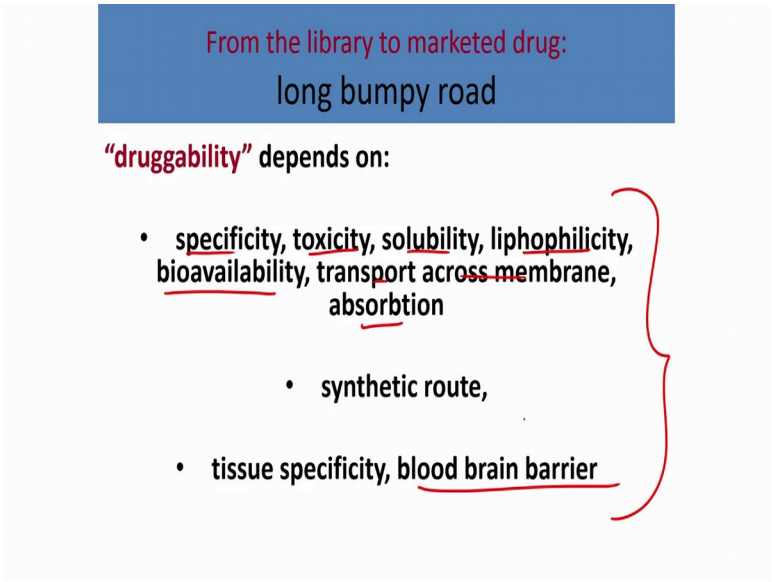

So, again this is a long bumpy road, the first thing you want to figure out is whether a drug is druggable and what that means, is all of these properties are actually being tested out. You want to make sure it is specific, the toxicity of it, what is the toxicity if any and the solubility how you are going to deliver it.

So, that includes the lipophilicity, how much you need, how it is becoming bio available, how is going to transport to different regions in the body, how it is going to get absorbed. And then again how it reacts with different tissues, are there synthetic routes to make it, is it crossing blood brain barrier. And a several more I mean this is just some examples I have listed here, but all of this needs to be checked out as you go.

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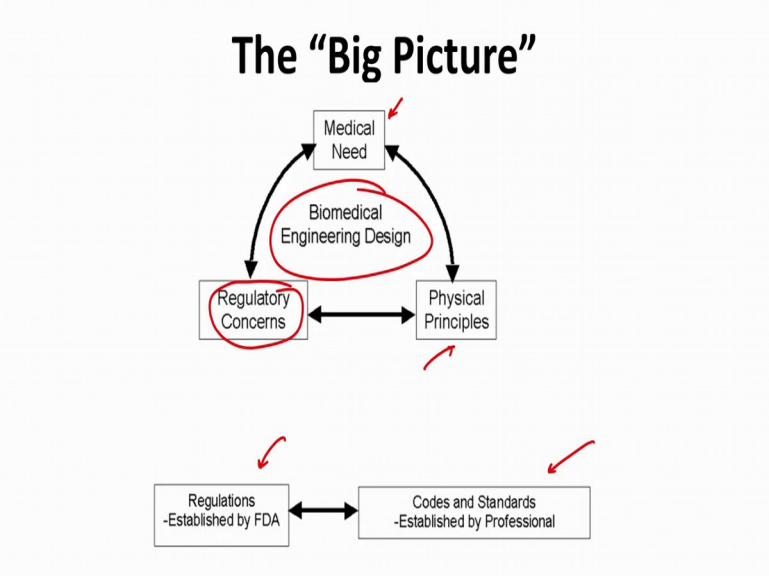

So, in the context of the big picture, you are looking at some kind of a biomedical engineering design centred around a medical need. But then you also have to consider regulatory concerns as well as some of the physical principles that is going to define that. So, there are regulations defined by FDA, ICMR various agencies and then there are several codes and standards, that are established by professionals that you will have to get the approvals through.

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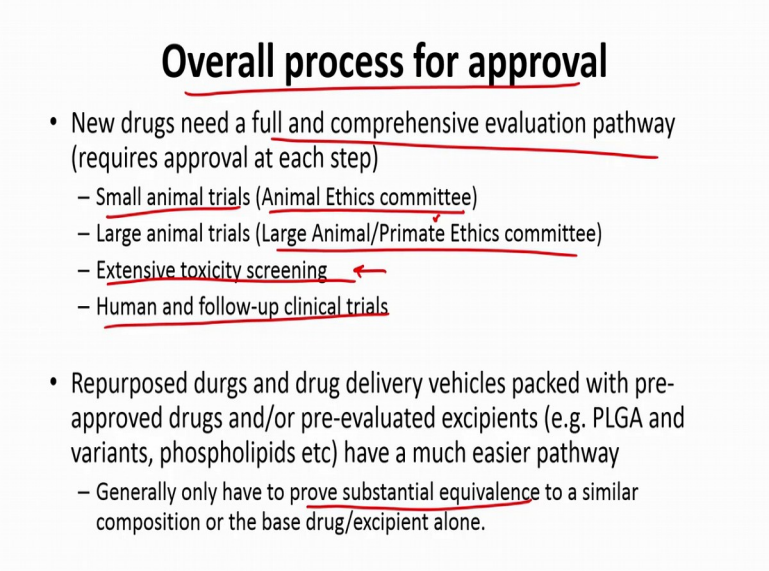

So, I will go again over the overall process for approval. So, if there is a new drug you require a full and comprehensive evaluation and there are approvals that is required at each step. So, if you are doing in a small animal trial you need an approval form as animal ethics committee to let you first of all test it on animals. If you are doing a large animal trials, you will need a large animal ethics committee, primate has a separate committee altogether. So, if you are doing trials on primate you will need those approvals.

Then if everything looks good before you go further you have to do an extensive toxicity screening to make sure it is not going to affect anything else. And then you finally, need human and follow up clinical trials and then we will need a human ethics committee approval. Just a quick note here if you are using a repurposed drug, let us say there is a drug that is already being used for cancer treatment and then your study shows that this drug may also be good for diabetes, then the approval is much easier because a lot of these toxicity screening have been done.

So, for example, a lot of polymers have been approved, so you do not have to individually test them, but you still will have to test them along with your drug of interest. So, and in general you will have to show in these cases substantial equivalence to a similar composition and to be able to get that drug to approve. So, it is a much easier pathway essentially if you are doing a repurposing of a drug.

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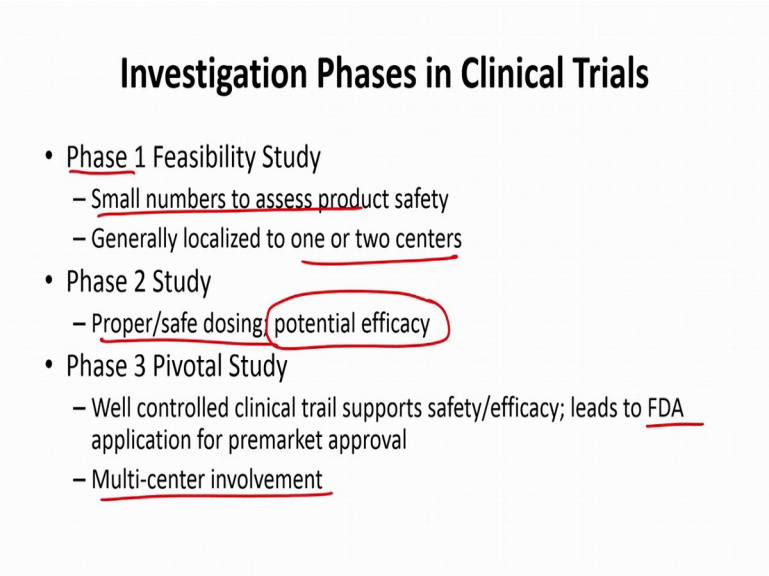

And then in human trials also there are the several investigation phases. So, what we might have heard very commonly, is called phase one clinical trial and that is to assess the product safety in humans. So, again clinical trials are divided into different phases and the phase 1 is usually a small number to assess product safety.

So, it is generally localized to one or two centres, so maybe you tie up with one of the hospitals in the surrounding or maybe it is coming out from the hospital itself. And they will get proper approvals and maybe recruit 10 to 20 patients and just see, the injection of this whether it is feasible or not.

Then there is a phase 2, trial which is slightly bigger than the phase 1, which is to do a proper safe dosing to figure out how much dose you can give. And also look at potential efficacy not very deeply into the efficacy, but mostly to see the safety and the dosing of that. And then there is a phase 3, this is a pivotal study, this is a well controlled clinical trial that will support the safety and efficacy, this could lead to an FDA approval or any other similar regulatory agency depending on the country.

And it is usually a multicentre involvement there are several centres there are involved instead of being one or two centres you may need to do it throughout the country to show that it actually first of all works in different settings as well as you may have differences in types of ethnics of the people that are coming in.

Some may be slightly different in certain regions of a certain country and the people may be different from the rest of the regions. So, you may want to instead of giving overall approval by testing only on the one set of the people you want this tested on how will satisfy people that is why the multi centre involvement is required in such cases.

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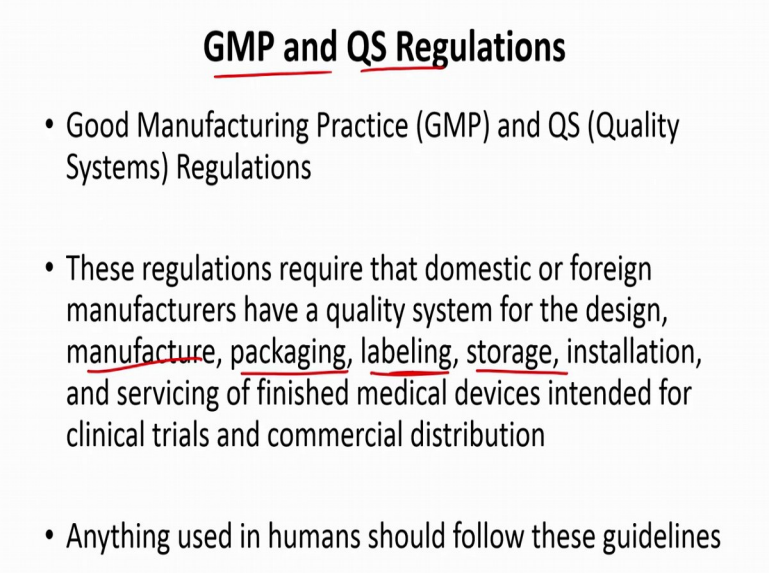

So, having done all that and again this whole process takes quite a bit of time depending on the disease and the extensivity to it, this could take anywhere between few years to 5, 6, 7, 8 years for the all the human face trial to get completed. Not to mention the amount of time that you would have first put in to take a drug, test it in vitro, test it then in animals and only then go to humans.

So, we are talking about almost a decade time frame to get all this data. And then let us say even if that all is done then we have a GMP quality system regulations, and these are good manufacturing practices regulations. So, you want to ensure that if anything is being put into humans these have been synthesized using some good manufacturing practices, it is sterile, it is not contaminated, so all of those factors then also come into play.

So, these regulations require that domestic or the foreign manufacturer have some quality system for the design. It is not like you can make that anywhere, you need to make that in a certain contained environment and making sure that it is properly manufactured, it is properly packaged, it is very properly labelled.

Again very important and quite a lot of debate these days. it is stored properly you have a system in place to install it and use it for different kind of clinical trials. And anything that is going to be used in human will need to follow these guidelines. So, even for the

phase trials the phase 1, phase 2, phase 3 all of this first needs to be established before you can go to those trials.

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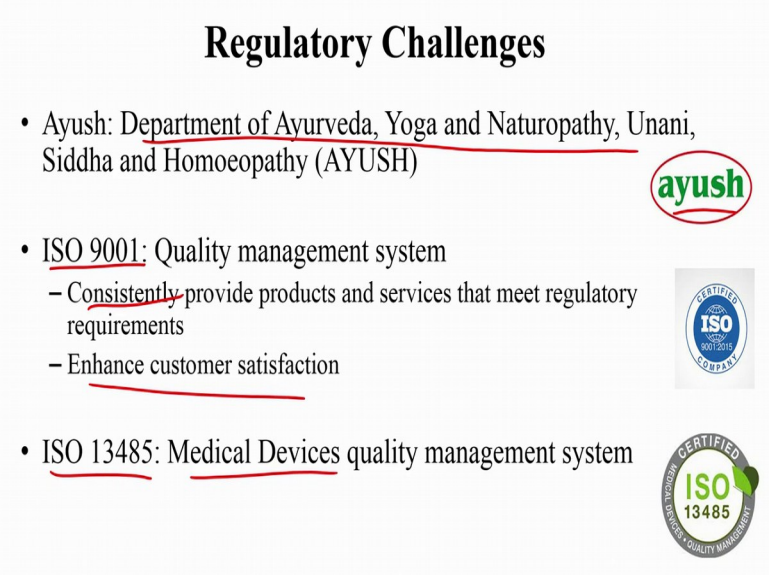

And then there are as I said the regulatory challenges, so you will have to get approval depending on, so this is more India specific this will depend on how much, what kind of product it is and how the regulatory agencies classifying this. So, you will have to get certificates from these agencies, so, Ayush is nothing, but a department of the Ayurveda, yoga, naturopathy, Unani homeopathy. If your product lies in this category you will have to get approval from them.

Similarly, there are various standards such as ISO 9001 and ISO 13485 and so these are both quality management systems, and defined for various types of things. So, this could be a medical device or this could be something else and they are here to sort of make sure that you are consistently providing products and services that meet these regulatory requirements. So, these agencies are going to check to see if you are satisfying a certain criteria and only will then certify you. And eventual goal is to enhance the customer satisfaction as well as safety.

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So, this is all I had to talk about in the course, I just going to give you a quick overview of what we do. So, I am Rachit and here is my email ID and twitter handle, if you guys ever want to follow me. Feel free to contact me as required, our lab does quite a lot of biomaterial work. There are tissue mimics and vaccines as well as the osteoarthritis repair and lung infections. And we have several positions that keep coming in the lab the final year projects, the PhDs and post-docs, so, please feel free to apply.

Basically, I hope you would have liked this course. That is all we got for this course. I look forward to interact with all of you. And feel free to reach out to us if you have any questions on this course and any comments, all of those are welcome. So, I hope to see you all and good luck for your exams.

Thank you.