Medical Biomaterials
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Lecture – 22
Animal Studies

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Hello every one. Welcome to the course on medical biomaterials. We will continue on the topic of animal studies. The most important thing in animal studies is the animal ethics especially when we are doing experiments with animals, we need to look at the ethical issues and we need to follow those ethical guidelines. Generally, any research organization if they have an animal house, they will always have animals ethical committee. So, this committee will look at what type of experiments are going to be performed, and whether they are abiding by the guidelines and so on actually. So, they have to get permission from the animal ethical committee before experiments are carried out in with animal.

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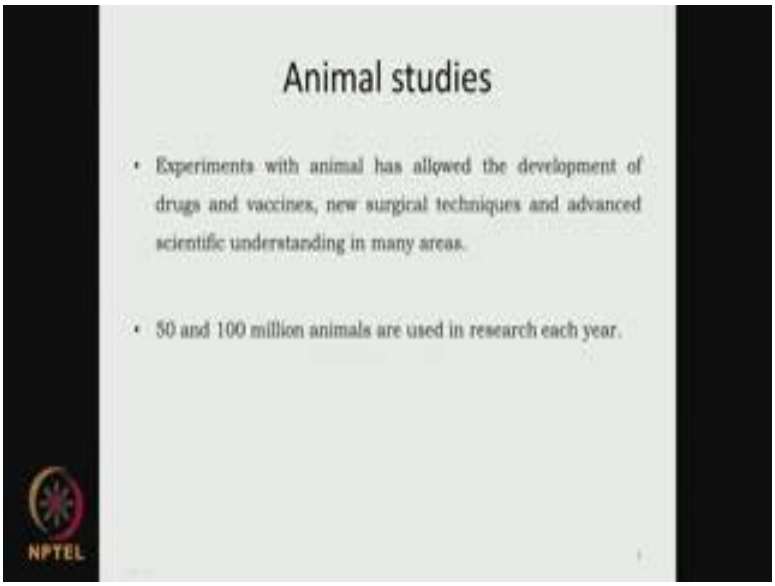

So, what are these? Animals are allowed for development of drugs and vaccines. And animals are used before they always going to human volunteers trails and even when the clinicians or surgeons are looking at new surgical techniques trying to understand certain concepts. Then they have to perform on animals this. Animals like I said in the previous class it could be small animals large animals right. Sometimes going ride up to horse starting from mouse. So, depending upon the type of animals studies they want to do.

So, quite a lot of animals are used globally for research per year and many countries have the mandate that animal studies have be done before they are taken into human volunteers.

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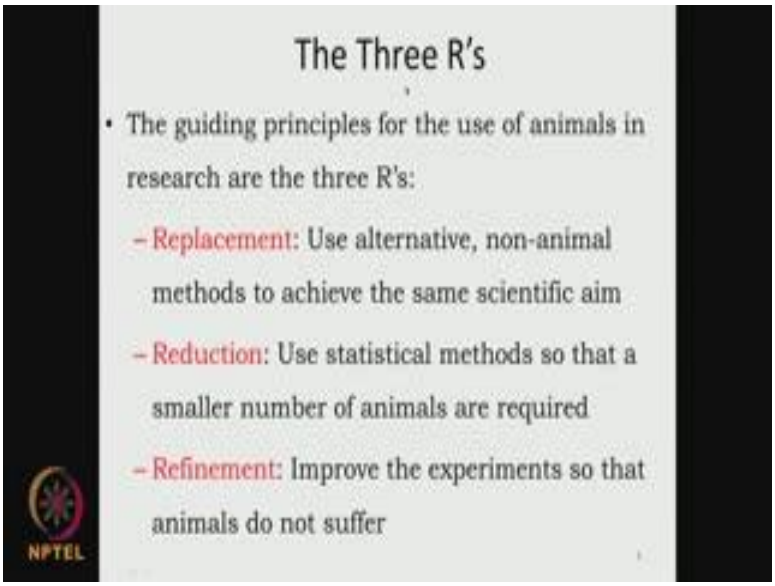

There are 3 important guiding principle, when you are looking at animals they are called 3 R's in animal research. One is called the replacement, another one is called reduction, other is called refinement. What is replacement? We need to understand whether there are any alternate available, which chooses non animal study. For example, can I use cell lines can I use computer simulation techniques and so on. Of course, computer simulation techniques have not developed so much that it can completely replaced animal studies and similarly cell line are not completely replica of animals. So, we need to still do animal studies.

Reduction, can we use less number of animals, but at the same time get statically significant information. So, can I use smaller set of animals? Can I do an implant on 2 sides of the same animal that number of animals can be reduced. So, you need to think of all those. That is called the reduction. Refinement can I improve the experimental strategy. So, that the experimental strategy is well refined. So, that the animal do not under go to much of pain, they have minimal pain the experimental duration could be short and quick and data is collected in a very efficient manner that is called refinement.

So, the main guiding principle in animal testing is the replacement reduction and refinement. So, any ethical committee will ask this questions can you reduce the number of animals, can you have experiments which does not use animals, or you refining your

experimental strategy. So, that they animals do not face too much pain or the experimental duration is much smaller and so on actually.

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So, there are lot of guidelines regulations. So, that the animals are treated humanly as possible that is very very important. Something called guide for care and use for laboratory animals, there are certain guidelines available the guide for care and use of agriculture animals in agriculture research and teaching, like sheep goat cow and so on. Report for the avma panel on euthanasia, in case animals have to be sacrificed how humanly it is possible without causing pain guidelines for the use of fish in research, American guidelines which looks at animal care and use policies, animal welfare act regulations public health service policy. So, there are lot of guidelines available both in Europe and as well as in USA for treating of animals of various types for they euthanasia and so on actually.

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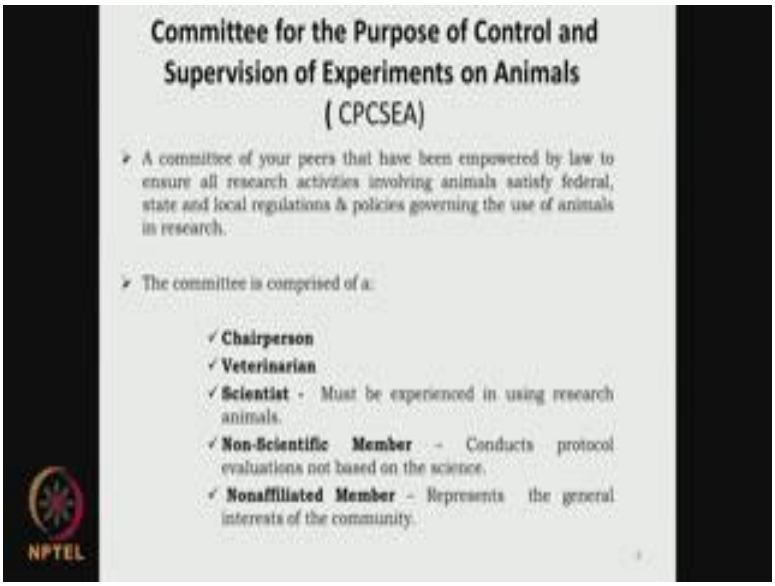

So, there is something called a committee for the purpose of control and supervision of experiments on animals. This committee I am sure as to be there in any organization which handles animal which conducts test of various types. We drug discovery where biomaterial studies and so on actually. So, this is the CPCSEA this a committee. That have been empowered by law to ensure all research activities involving animals satisfies the federal state and local regulations and policies.

Both governing the use of animals and research, so that, they have the mandate to look at all the research activities that are going on with related to animals. So, there is a chair person there as to be a veterinarian here doctor who is an expert in handling of animals scientist, who has done research on animal then there as to be nonscientific members. So, nonscientific members look at it at different angles and tells whether the experiments that are going to be conducted is really important, whether the animals face pain and whether it is done humanly and so on nonscientific member. Then there are non affiliated member; that means, people who are not affiliated with the research organization, outsiders who are more interested in the community as a general community or large.

So, people who are looking at as a community based issues. So, those are also members who are inducted into this particular committee. So, we have a chairman we have a veterinary surgeon we have scientist who is going to be conducting the experiments who is very well experience. Then there are nonscientific members who does not have much

science back ground, but they may have other back ground and then members who are inducted from outside the organization. So, they are more interested in the welfare of the community at large. So, this all people from this committee and they go through the protocols or they do experimental strategy which is designed and they try to understand it and the use all the 3 important principles 3 R and then makes suggestions which are implemented before the experiments are started.

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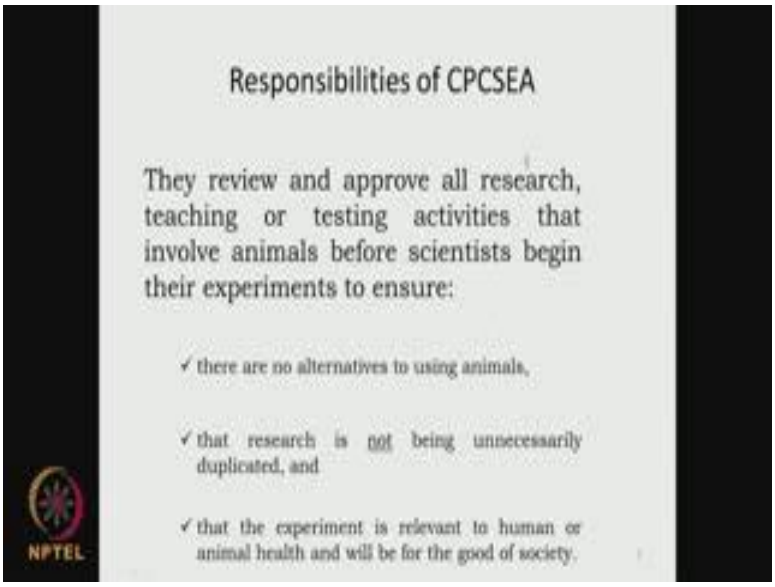

So, what are the responsibilities of CPCSEA? They review and approve all research, whether it is a research teaching or testing activities that makes use of animals small animals large animals and so on actually. They look at whether there are no alternatives to using animals and the research is not duplicated. If the data is already available why carry out the research. So, they might not approve if it is a duplication. And they also look at whether this experiments are relevant to human or animal health. So, it as to have some purpose especially may be if it is drug for human or it is a drug for animals. So, you need to test it out approve before actually tested on human or animals. So, whether it is relevant on it is good for the society.

So, it is not that I want to do an experiment just for the sake of doing experiments, but there as to be endpoint where the society at large comes into picture. So, we need to consider that angle as well. So, the CPCSEA looks at all this aspect before the actually give permission to the organization for conducting the experiments.

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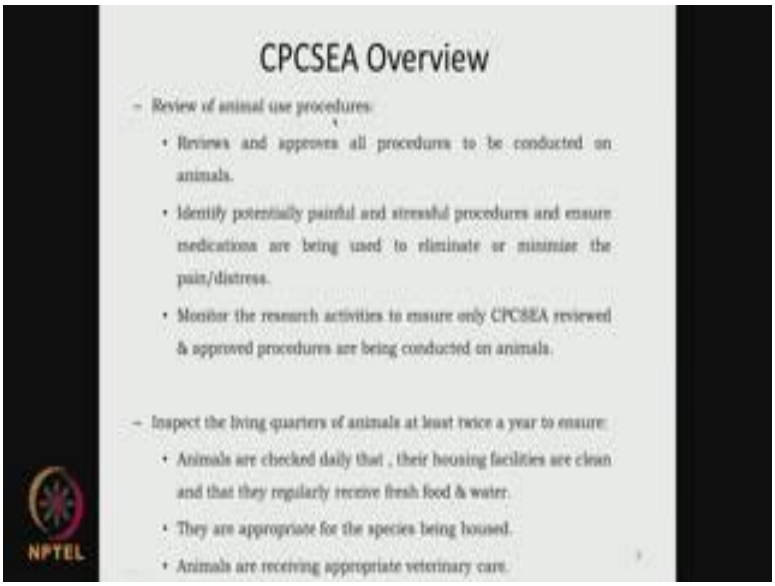

So, they look at the procedures. So, they look at each and every protocol and then approve it to be that conducted on animals. And then identify potential pain full and stress full procedures. So, if there are going to procedures are going to pain full can animals be given medication or can we completely eliminate this procedure or can be minimized this pain and this stress. Then also monitor the research activity and ensure only whatever as been reviewed and approved by the CPCSEA followed. So, it is not that by the experiment does something different from models of approved by the CPCSEA.

They also inspect the living quarters of animals may be twice a year. They come and see whether the animals and have a good facility animals are checked daily there housing facilities are clean and the space for each animal is sufficient for there wellbeing as well as for the production, that they regularly see fresh food water. There are appropriate for the spices being used. For example, if it is rats there are certain guidelines in which they have to be housed, if it is guinea pig there are certain guidelines if bigger animals like dog or sheep or goat then there are certain guidelines for their housing whether food and water. So, whether they are followed and they are receiving proper veterinary care that is also important. So, there as to be a full time veterinary surgeon who takes care of the wellbeing of this animals, so that is the overview of the CPCSEA.

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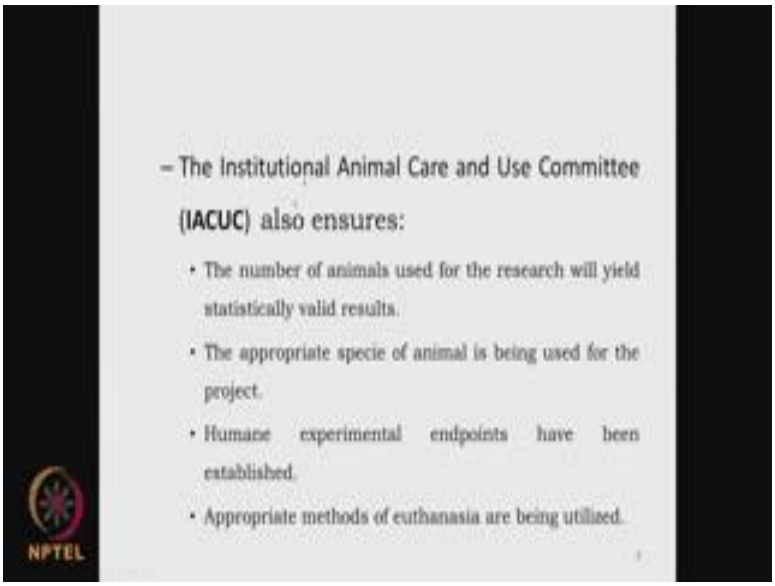

Then of course, there is a committee that is called institutional animal care and use committee IACUC. So, they look at whether the number of animals are enough to yield statistically valid results. So, neither want to have too many animals nor too little animals which are not statistically significant whether the appropriate specie of animal is being used for the project like I told you some time back. If you are talking about cardiovascular stent what type of animals, if you are looking at joint what type animals if you are looking at just cytotoxicity what type of animals and so on. So, the appropriate specie of animal and humane experimental endpoints have been established that mean there as to be start and end.

We are studying the bio degradability of a polymer over a period of 30 days. So, that is the start and end point. So, that is also very important. So, there has to be end point. So, it is not an open ended research which they are doing. And then whether they are using appropriate methods for euthanasia if the animal is scarifies. How it is being done what procedures are being done. So, that the animal does not face pain when it is sacrificed. So, all this issues needs to be looked at and which is being looked at by the institutional animal care and use committee.

So, there are quite lot of overseeing committee, which not only monitor the wellbeing animal, but they also review the experimental procedures strategy they give lot of suggestions modifications, with respect to the 3 R and if there is any animal as to be

sacrificed how painless, they have to be handled and so on. So, this committee take care of all this issues.

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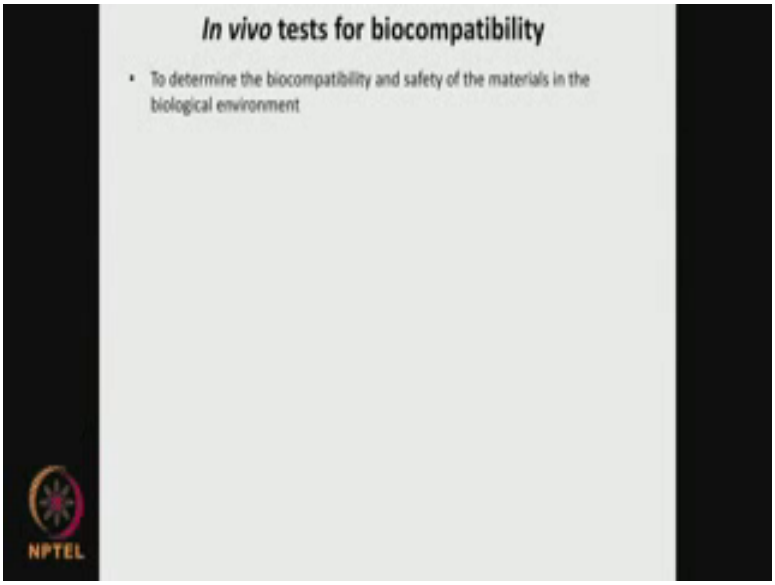

So, let us look at if a couple a few case studies, and which are we have performed using animals looking at biomaterial with respect to their biocompatibility or bio degradability. So, imagine I am looking at the biocompatibility of certain polymeric system in vivo I might have apriori done with cell lines I talked about an essay called mtt or mts assay, which look at a self proliferation and so after doing that you want to move to the animal and look at the biocompatibility and biological environment, because when you when you keep it inside the animal it is facing enzymes proteins and so on. So, the way this material behave they may be very much different the way the in cell line.

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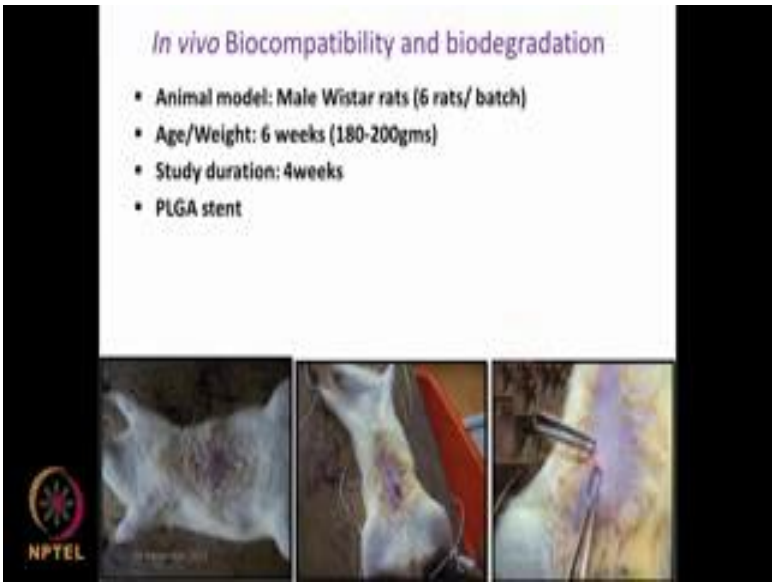

So, we have done some experiments I want to show you this is we are looking at in vivo biocompatibility and bio degradation of a polymer called poly lactic glycolic acid, which is as supposed to be an alternate use in stents urethral stents currently poly urethane is used and so we want to look at this PLGA in animal model male Wister rats we have 6 rats per batch, so control and the test and so on. So, the age weight about 6 weeks and one 80 to 2 hundred grams, so the polymers are placed intraperitoneal and then as showed back and after 30 days, they are removed and then the biodegradation of the polymer can be studied by using weight loss and by looking at the tissues one can look at whether the polymer a causes any cytotoxicity, or whether it is harmless to the surrounding tissues and also look at systemic toxicity whether the polymer those 30 days causes toxicity system is well.

So, this type of cytotoxic studies this are very normally done .and you can do it for short period or medium period or long period like I said around 30 days is what studied if you are taking about longer duration studies. So, after 30 days you take out the sample out which is placed in intraperitoneal and look at not only the changes when the polymer you can also look at any change systemic toxicity to the rats as well then you can take out certain tissue samples tissue sample from the heart.

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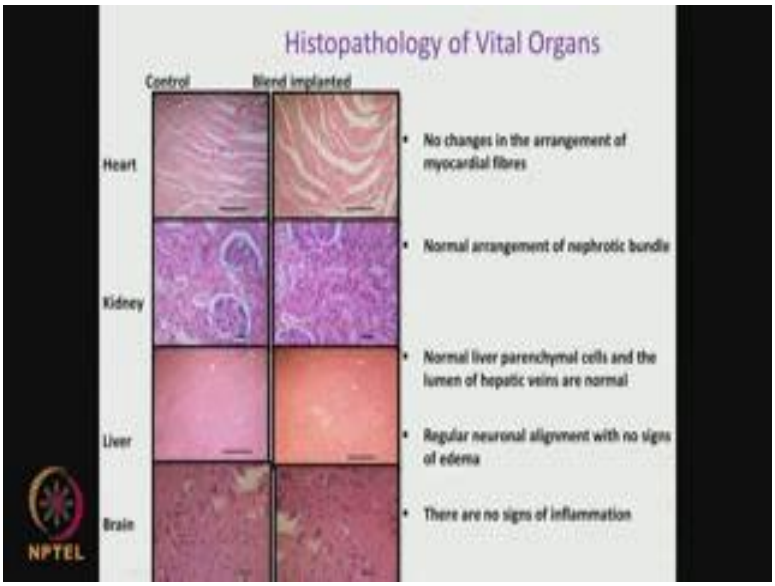

And then see whether there is changes after it is being placed the polymer for 30 days. So, you can prove there is no changes in arrangement of myocardial fibers, we can take out kidney samples and then we can see whether there is a any changes with respect to control, we can look at the liver sample and then if the control as well as with polymer placed and see whether any changes as happen. And also we can take tissues from the brain and see there are no inflammation and so this type of studies are commonly done and to look at the toxicity both the acute as well as chronic and also systemic toxicity. So, we take tissues from different parts of the animal and see whether there is a change because the material has been placed for 30 days intraperitoneal with respect to the control.

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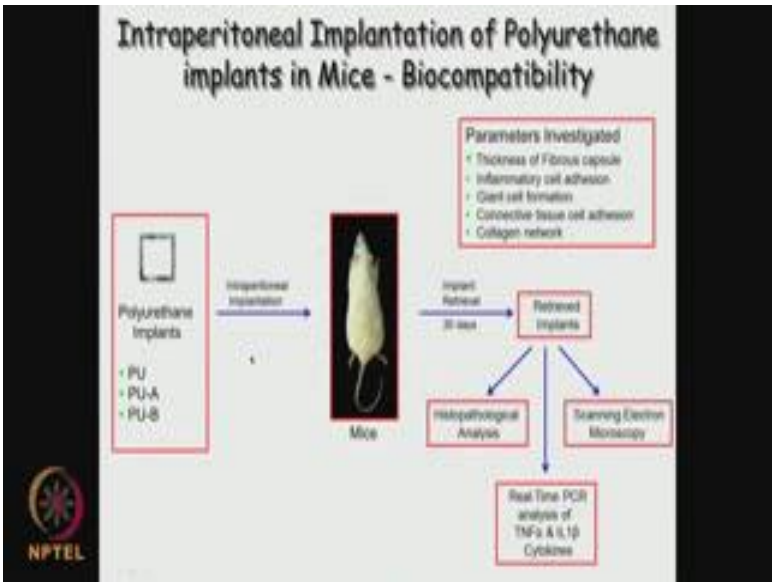

So, similarly that is with respect to inflammation, then we can also look at whether there are any gen cells that have been formed, whether that are connectivity tissues have been formed whether there is any collagen network that has been formed and so on. Again here I am showing you an example where we have looked at polyurethane. Polyurethane is widely used in stents they are used in breast implants they are used in many places. Actually in tubes and so on actually.

They we have 2 different modifications that are done to polyurethane they are placed intraperitoneal for 30 days. And then we retrieved implants look at the histopathological study we can also look at fibrous and capsulation around that polymer is there any collagen as been formed in the previous study we talked about inflammation here, we are talking about what are the changes that are happening to the connective tissues tissue respond.

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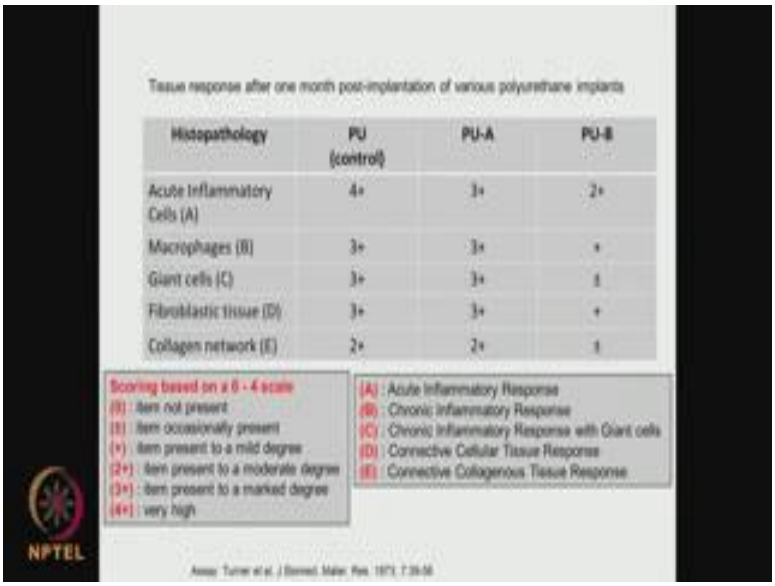

So, we can sort of form the histopathology data. We can count the number of tissue different types of tissues are formed and then we can sort of quantify them here. This is the control this are 2 different 2 modifications to the control after, one month of implantation we are looking at different types of tissues that are formed acute, inflammatory responds macro phages joint cells and fibroblastic tissue collagen network and then we look at each one of this polymer effect each one of polymer this polymer.

So, we say when we give a scale of 0, we say it is not present when we give just plus or minus we say it is occasionally present, when it is present then we say it is present to a mild degree, when we say 2 plus then it is present to a moderate degree 3 plus will say marked degree 4 plus very high. So, this is how it score basically check it is a qualitative representation of the observation. So, this is the control we say acute inflammatory cells it is found to a high very degree, where as in the modified system which found to mark degree in one modification is found a moderate degree.

So, there is a decrease in the inflammatory cells as we modify the control polymer macro phages it is found in mark degree on the control, and then it goes to down to almost very mild degree; that means, among macrophages found on the animal model on this modified system goes down from a mark degree to very mild degree. If you look at the giants cells it is found quite a lot on the control, but it comes down quite drastically and which is say occasionally found. Same thing on fibroblastic tissues from a mark degree it

comes down to almost mild and collagen network it is found to moderate degree to almost to occasionally present. So, effect of this modifications with respect to the control is sort of determined in a very qualitative way, and it gives you a very nice picture on the formation of various types of a cells network and inflammatory responds. So, this is a very interesting study one can do using animal models and as you can see we cannot do study in a in vitro cell line.

We can look at the inflammatory markers like TNF alpha or IL beta which I showed you, because of the contact of the cells with biomaterial, but if you want to look at fibroblast collagen network giant cells then; obviously, we need to do some type of animal studies we cannot do it in vitro condition. And we can do some qualitative configuration and as you can see in this table modification to the polymer brings down this tissue responds dramatically with respect to the control. So, tissue responds we cannot study in cell lines, but you have to study of course, in vivo model like a Wister rat.

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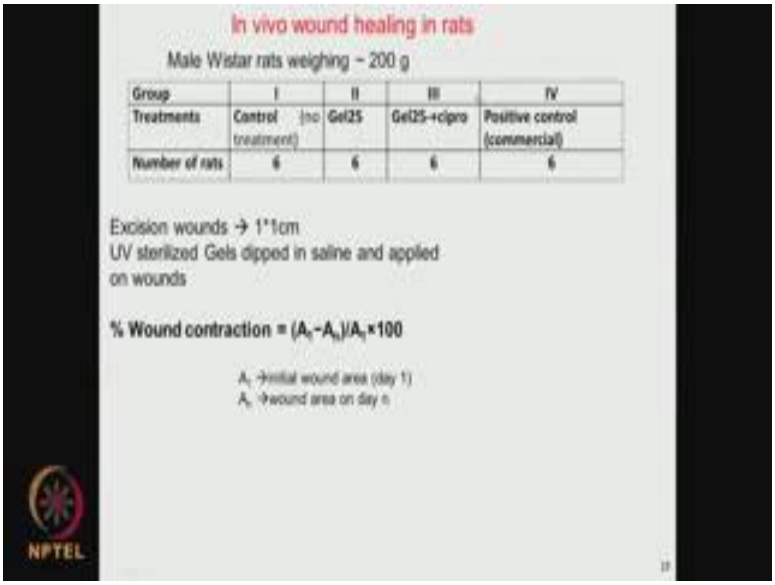

Another example I want to show you are looking at wound healing. Develop polymeric material for wound healing applications. So, we have create wound on the surface of rats and then we look at how the various polymeric combinations blends, this is a polymeric combination gel 25 using a glucan and a carrageenan where 25 percent of it cyclic glucan remaining is carrageenan here same polymer, but ciprofloxacin which is an anti bacterial when you have an open wound bacterial infection could be a problem. So, we can see

whether ciprofloxacin in preventing bacterial growth and this is a commercial product as a control.

So, we have a control where we do not give any treatment then we have a combination of a cyclic glucan and carrageenan hydro gel, treated wound and this is same thing with the ciprofloxacin and this is a commercial product. So, here we are talking about 6 rats in each case. So, here we create an wound on the surface and then then this different treatment are given. Then is monitor how the wound area contract as a function of time you do this experiments for 7 days. And then see how it contracts as a function of time and then we see whether there is a statistical improvement, because of this type of new polymeric design and that is what one does actually.

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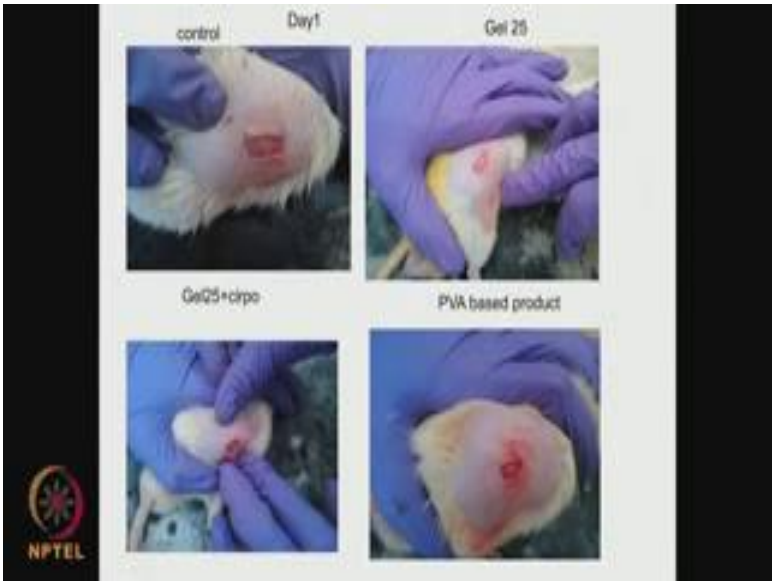

So, this pictures shows you the wounds on control without treatment with the carrageenan, and cyclic glucan this is with carrageenan cyclic glucan and ciprofloxacin this is a commercial product and so on with different times periods day 1, day 4, day 7 and so on.

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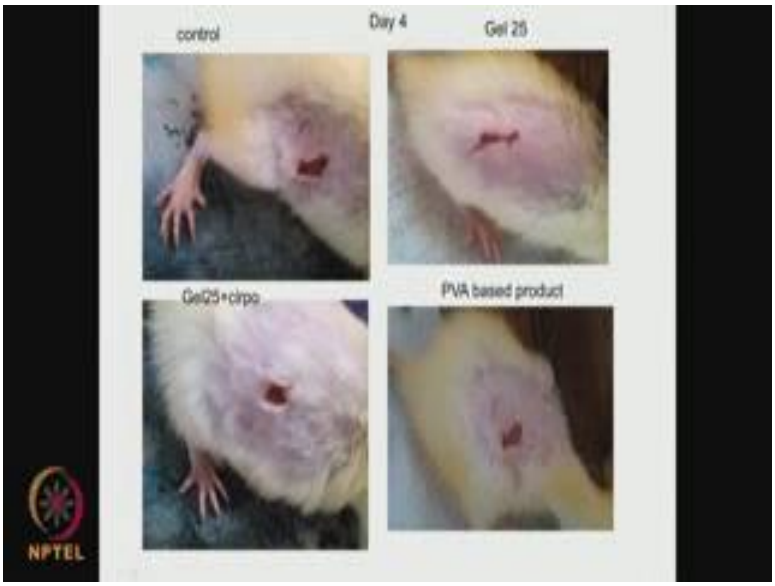

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We can quantify the wound contraction area and represented beautifully in this type of bar graph. So, what does it tell? As we go up in days this is with the ciprofloxacin hydro gel this as without ciprofloxacin, this is a commercial product this is the control.

So, we can see wound contraction is very high very fast. Within 2 days we find very high wound contraction when compare to the control or with commercial product by the contraction rate increases dramatically within the 8th day, when compare to the control the wound contraction after 8 days in control is only 60 percent or 70 percent where as it

is almost 100 percent. The wound it completely yield as you can see here in day 8 this is the day 0, and wound is still not yield fully in the control where we do not give any treatment. So, we can see this this is with ciprofloxacin (Refer Time: 24:42) and this commercial product. So, this particular hydro gel seem to work regionably well, and this type of experiments are carried out and have to be carried out this type of animal models without that one could not go further for testing it on human.

So, we can also look at in addition to the wound contraction we can look at whether the collagen network formation is good. And so that the wound gets completely yield because that is more of a tissue responds like I been telling for a long time. So, you have a inflammatory responds then you have tissue responds and so on. So, the major constituent of collagen it is called hydroxyproline. So, we can monitor taking out samples from here what is the hydroxyproline content which is a marker biomarker it is said which tells you how the wound gets contraction rate is good.

So, as you can see here hydroxyproline content is quite good and with compare to the control or with ciprofloxacin without ciprofloxacin we see a quite big increase in the hydroxyproline content with respect to control; that means, the network of collagen is also growing one is the wound contraction and then the collagen network also growing very well and so the wound gets completely cure. So, the hydro gel treated wounds are moist no sign of infection and inflammation and the healing rate is also very high and almost more than 90 percent in 8 days, when compare to the control or commercial product on the high level of hydorixyproline, which gives you idea about the increase in the collagen build up and coverage of the wound is also very high with respect to the control.

So, I given you few case studies or examples, where use of animals small animals is extremely important before one goes into human volunteers. This studies which I showed you with small animals mostly looking at cyto toxicity, we can look at biodegradation of polymer we can look at the wound healing properties, we can look at the tissue formation and whether there are any collagen network formation when a material is implanted intraperitoneally. Of course, you need bigger animal if you want look at joint replacement or if you want to cardiovascular stenting, because the rats have very small sized joints as well as wains. So, we may have to go 4 higher animals like dogs sheep and so on, if you want to look at other type of studies. So, the rats, Wister rats or rabbit is

they are extremely good for the types of studies, example which I showed you like such as cytotoxicity such as biodegradable, such as formation of collagen network, such as inflammatory responses wound healing and so on actually.

Thank you very much for your time.