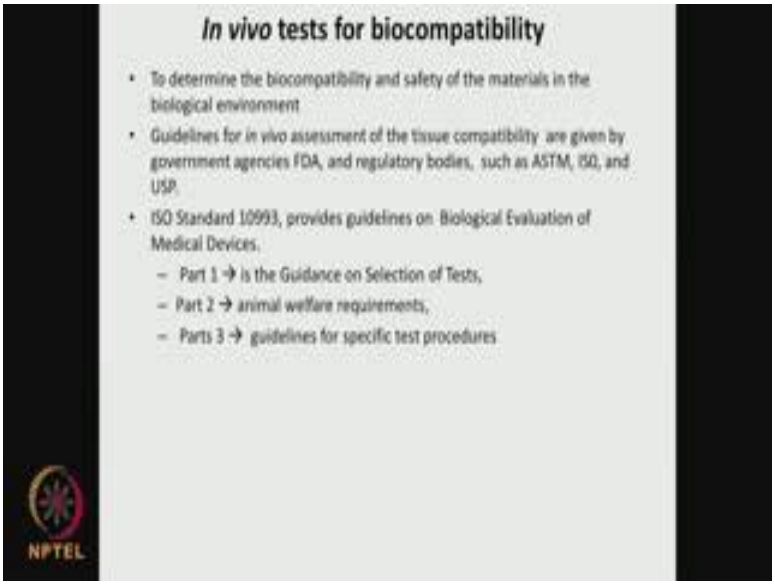
Medical Biomaterials
Prof. Mukesh Doble
Department of Biotechnology
Indian Institute of Technology, Madras

Lecture – 21
Animal Studies

Hello everyone. Welcome to the course on Medical Biomaterials. Today we are going to talk about animal studies; that means, in vivo studies and animal studies are supposed to be carried out before they actually test it out on human volunteers.

(Refer Slide Time: 00:28)



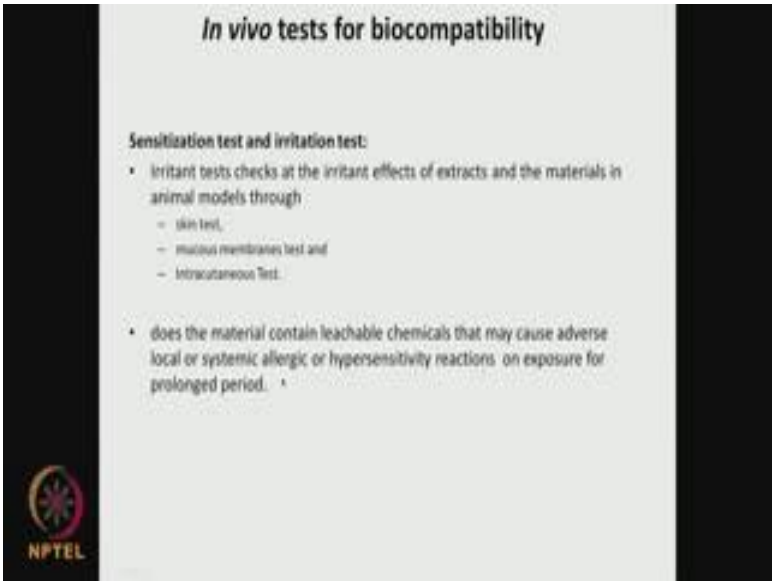
So, you want to look at the biocompatibility of the material under in vivo situations; that means, you want to look at biocompatibility safety in biological environment whatever we do in the cell culture lab in vitro nothing like performing it in animals because a animals are the real system where we have a lot of enzymes we have the blood we have the other body fluids taking part.

So, the way the biomaterial behaves in such environment may be completely different the way it behaves in very controlled in vitro there are guidelines for in vivo assessment of the tissue compatibility given by FDA regulatory bodies ASTM, ISO, and USP. So, there is ISO standard 10993, it provides guidelines on biological evaluation of medical devices if part one gives the guidance of selection of test part 2 gives the animal welfare requirements, part 3 gives the guidelines for specific test procedures.

(Refer Slide Time: 01:32)

Now, if we look the declaration of Helsinki in 1964, it mandated that medical research on humans must be supported by preceding animal research. So, before it goes into human volunteers there as to be experiments conducted on animals. So, this has been taken very seriously and they made it into a law in USA by the FDA and Europe by the European agency for the evaluation of medical products. So, animal trail is emerged before it goes into the human volunteers.

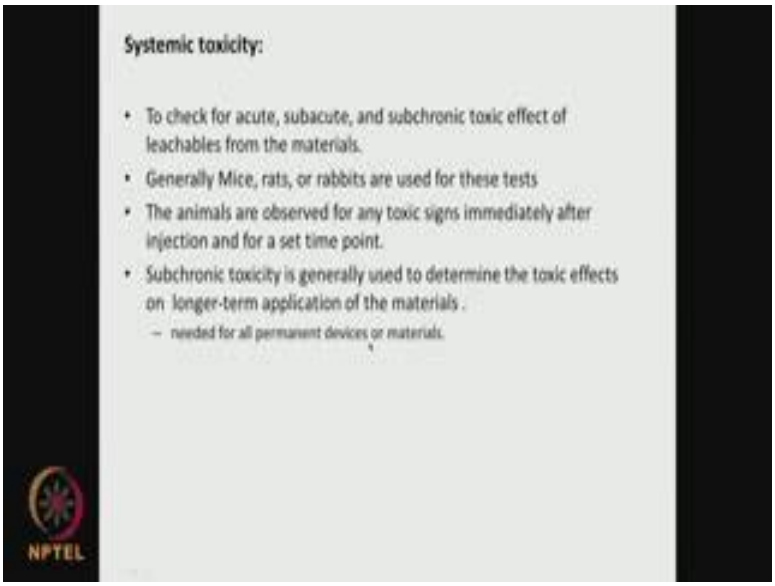
(Refer Slide Time: 02:09)



So, what are this in vivo test for biocompatibility that is something called sensitization test and irritation test; that means, does the biomaterial causes some irritation to the animals does it become very sensitive to certain body parts or tissues it is not only the material, but also the extracts from the material.

So, things like skin test mucous membrane test intracutaneous test. So, there are different types of test basically looking at whether it causes irritation sensitization to the animals so; that means, that does the material contains leachable chemicals that may cause adverse local or systemic allergy or hypersensitivity reaction on exposure for prolonged period. So, that is what is called sensitization test and irritation test.

(Refer Slide Time: 03:06)



Then comes systemic toxicity does the material give over all toxicity to the animal like acute toxicity subacute toxicity; subchronic toxic not only the material, but also the leachable. So, you need to understand it is not only the material, but whatever its getting leached out from the material also as to be studied in detail over all this in generally mice rats or rabbits are used for this systemic toxicity test actually the animals are observed for any toxic signs immediately after injection and for a set time point for. So, many hours short duration long duration. And then one studies whether it causes any toxicity to the system.

Then subchronic toxicity; it is generally used to determine the toxic effect on long term application one is short other is long term especially we need it for permanent devices

like devices which like stents for example, or orthopedic implants or new joins die from valves heart pacemaker all this are going to be permanent devices. So, the materials involved are they going to have any (Refer Time: 04:24) subchronic toxicity. So, short term toxicity long term system toxicity.

(Refer Slide Time: 04:31)

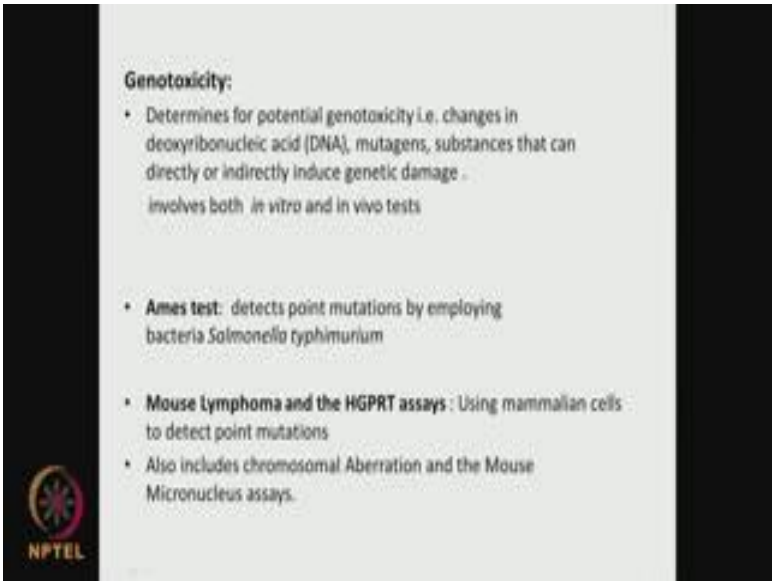
So, the various in vivo tests for biocompatibility include sensitization test irritation test acute systemic toxicity, test subchronic toxicity, genotoxicity, does it cost any problem for the gen and implantation test that is the material still perform and it is inside then body hemocompatibility does the material cause any problems to the blood or is it compatible with the blood carcinogenesis does it produce any cancer or carcinogenic effect to the animal.

Does it cause any reproductive toxicity or developmental toxicity this are much longer we look at the next generation of a animals and one need to look at it. So, pharmacokinetics this is very valid especially when you are talking about drug delivery system how fast the material goes from they; for example, the oral into the blood stream and how fast it gets eliminated. So, mostly it is for drug delivery systems, but not for permanent implantable systems or if there is a biodegrade able polymer how fast it gets degraded and disappears from the body preclinical safety testing. So, all this are histopathology then later on the various tissues from the animal is taken out it could be related to the kidney liver various body parts like heart and then one doctor pathologist

look at changes and doctor also looks at there are any inflammation that is caused of this material.

So, these are large number of test and you need to remember that it is not that you need to perform all the test for all the biomaterial. So, some biomaterial may require some tests some other may require some other test.

(Refer Slide Time: 06:40)



So, what is this genotoxicity? So, you need to identify does the material lead is a potential genotoxic effect that is changes in the DNA does it mutagens some gens. So, that substance directly or indirectly induces genetic damage that is what is called genotoxicity; that means, the material changes the genetic makeup either directly or indirectly. So, this may involve in vitro I can do it cell lines and I can do it on animals also then comes Ames test it detects point mutations by employing this particular bacteria called salmonella typhimurium.

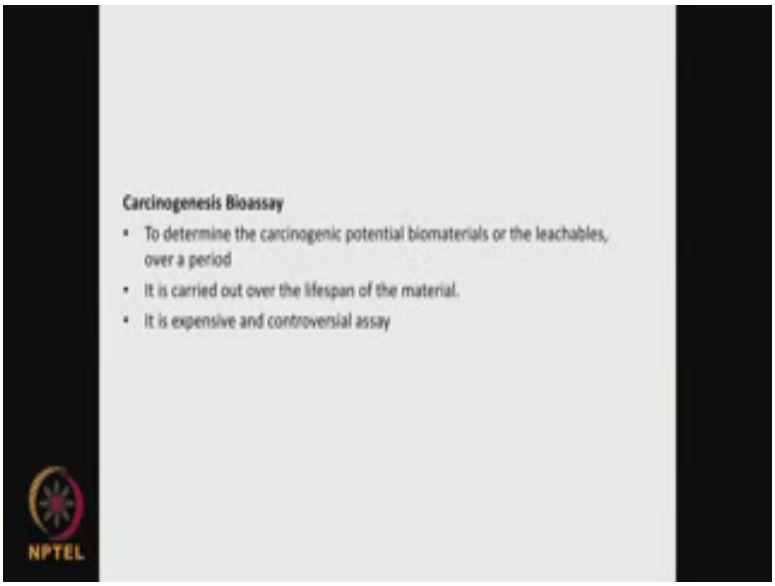
So, if there are any mutations when the gen at which place it got mutated and because of that what could be long term effects that is called the Ames test then look at the lymph's changes in the lymph pattern and this are called you can do it using a mammalian cells to detect the points mutations this are related to the gen it also includes chromosomal aberration and changes in the mice micro nuclear mouse micro nuclear assays. So, this are animal trails as well as some of them could be cell line based work. So, all this lead to understanding whether the material causes genotoxicity.

(Refer Slide Time: 08:12)

Then comes implantation test material is placed inside the body in different places and one needs to look at whether it is biocompatible when it is directly in contact with tissue other than skin part of it.

So, if you are talking about sutures implantable material one needs to understand that. So, you need to also test whether the material performs its job both in the short term as well as the long term. So, various implantation techniques various location inside the body in which the material is kept and it studied this provides information on the safety of the material.

(Refer Slide Time: 08:58)



Then looking at carcinogenesis you are looking determining the carcinogenic potential of the biomaterial; that means, whether it will cause cancer whether the leachable cause cancer over a period of time. So, if the biomaterial is going to permanently kept; so, maybe you have to keep the material for a long period in the annual and then see whether going to cause carcinogenesis. So, it could be may carried out over the life span of the material it is expensive and it could be controversial also.

So, we are not sure whether what the results coming out of the animal studies can be extended to human as well as life span means do you really run it for very very long period of time and so on actually. So, it could be controversial.

(Refer Slide Time: 09:45)

Now there are lot of test are done as per the guidelines based on the intended use of the material. So, we have surface devices; that means, your skin patches or wound healing patches external communicating devices. So, like you are pacemaker these are externally communicating bio censers implanted devices like your cardio vascular stent urethral stent. So, surface devices means it could be in skin it could be in mucous membrane or it could be breached or compromised surface. That means, you make a very surface patch slightly deeper if you look at external communicating device like blood path indirect tissue bone dentin circulating blood implantable could be tissue bone blood all this. So, the duration could be less than 24 hours 24 to 30 day greater than 30 days. So, and generally if you look at if you look at papers and look at what type of studies people have done we will see either they would have done for 24 hours or they would have done for 30 days. So, limited is less than 24 hour. So, if we want to prolonged; you may run it for 30 days animal and then look at the effect or if you are looking at permanent devices you need to at least for greater than 30 days.

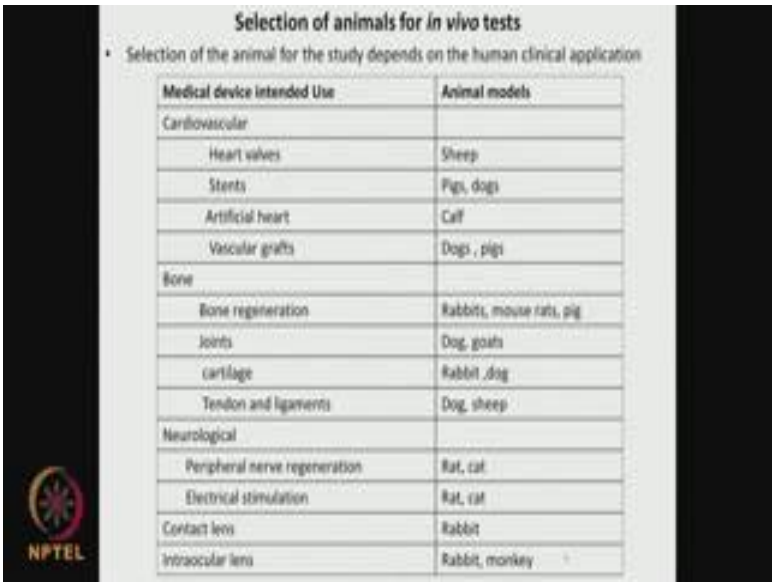
So, if you have different situations here ABC, ABC, ABC and so on and then you are looking at cytocompatibility, it is causing sensitization does it cause irritation especially if it is a very small duration or even if it is long duration in the skin, we are satisfied with these three different tests on animals please note this was this table was collected from biomaterial biocompatibility testing lab WHO, India if you are looking at slightly mucous then we not only do the irritation and sensitization we also have to look at

subchronic toxicity, genotoxicity, similarly if it is slightly breached we may again have to look at sub chronic and genotoxicity.

Now if you are going to have external communicating device; that means, the material is somewhere in the blood path then we may have to look at hemocompatibility also please note this and systemic toxicity even if it is a short duration or whether it is a long duration if it is a long duration we also have to look at chronic genotoxicity and hemocompatibility look at this. So, the number of test increases. So, if it is a skin test are mostly irritation sensitization if it is slightly longer duration we may have to look at chronic toxicity and genotoxicity, but if it is in the externally communicating device it is in the blood path we may have to do the hemocompatibility if we have to look at tissues then we have to do lot of other test systemic toxicity chronic toxicity genotoxicity then implantation test same if it is a blood related, then of course we do the hemocompatibility.

So, if its implanted device in a tissue again we can stick to irritation cytocompatibility in sensitization, but if it is down into the bone or blood we may have to do the entire set of test here; that means, not only the sensitization irritation cytocompatibility we have to look at systemic toxicity, we can look at subacute; subchronictoxicity, genotoxicity implantation test hemocompatibility. So, this is a very interesting table type of test that need to be done depending upon whether the material has to be for limited duration; that means, less than 24 hours for prolong duration; that means, 24 hours, 30 days or permanent greater than 30 days and whether it is a surface device whether it is an external communicating device or whether it is a implanted device. So, the number of type of test changes depending upon these 2 factors and these are the various types of test that needs to be done these are the guidelines that has been suggested by WHO India interesting table to look at selection of animals for in vivo studies.

(Refer Slide Time: 14:10)



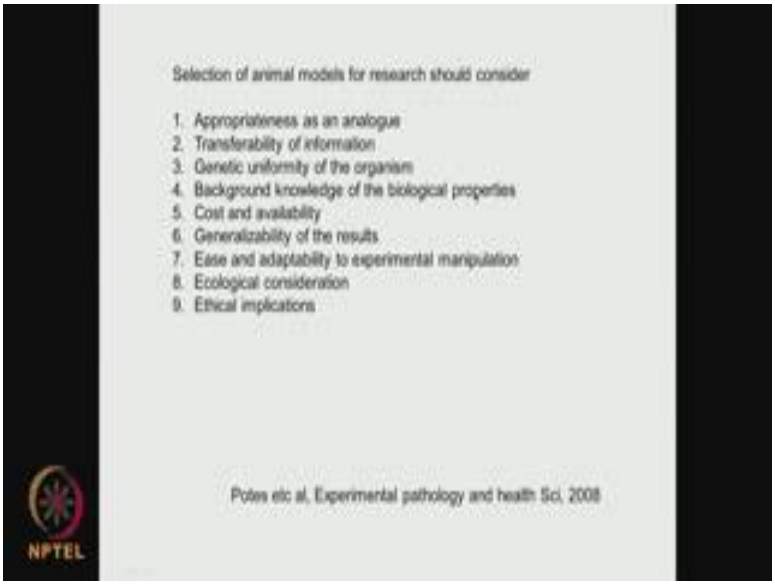
So, there are something called small animals there are something called large animals. So, small animals could be rats, mice, rabbit, hamsters; large animals should be pigs, sheep, dog, even horses and large animals are very expensive to work on small animals are much cheaper in some situations we have work on large animals because small animals have very small cartilages for example, if you are looking at cardio vascular stents the (Refer Time: 14:49) system is so small, we may not be able to do the stent studies in small animals we may have to go to large animals.

So, if you are looking at heart values we have to go to sheep we cannot do it in small animals stents again we have to go to pigs and dogs artificial hearts we have to go to really large animals calf's vascular grafts dogs and pigs bone, bone regeneration rabbits, mouse, rats. So, we can do in small animals, but joints we cannot do in small animals you see joints of small animals are. So, small we have to go to dog and goats cartilages we have to go to rabbit and dog tendons ligaments again large dog and sheep neurological peripheral nerve generation rats, cats, electrical stimulation rat and cat contact lens rabbit because eyes are quite small to test intraocular lens rabbit and monkey.

So, large set of animals are used in preclinical studies and depending upon whether we are looking at simple cytotoxicity bio degradability or if you are looking at the cardio vascular stents heart transplants or heart valve change or joint changes we may have to

go from small to larger animals, again this is a very interesting table to get a feel of what type of animal study that need to be done.

(Refer Slide Time: 16:18)



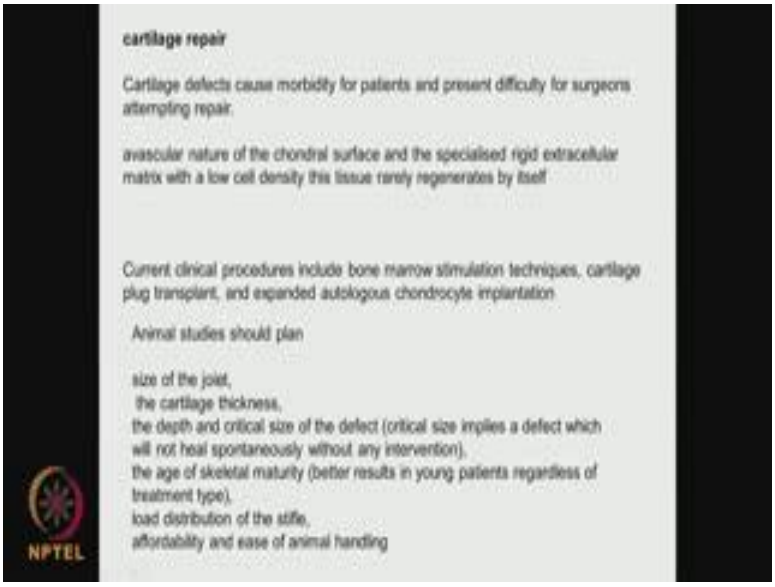
So, the selection of animal models depends on so many factors. So, this is taken from this particular reference appropriateness as an analogue is it appropriate to use this particular animal does they have is it correct to use that animal or it should be is the knowledge we get is too far away from the real human system transferability of information can we transfer this information I have got with this animal study to what I want to achieve later genetic uniformity of the organism.

So, if I am taking assay terrestrial rats genetically it will have uniform pattern genetic makeup back ground knowledge of the biological properties. So, we know all about for examples on the animal we have we may know all about terrestrial rats. So, immediately waster rats are used for many drug discovery programs because the biological properties are those are very well known for example, if in a bacteria, E. coli the biological properties of E. coli and the genetic factors are very well known. So, that is why in mini studies we will see E. coli is for example, is well known. So, many studies are done on east cost and availability cost plays a very important role if you are talking about animal studies with sheep and dog and horse it could be very expensive availability is it is easy available because when I am going to do experiment I may have to do on many samples. So, that is statically significant.

So, the availability also important general; generalizability of the results so whatever results I get, can I generalize it for many other conditions? I might not be able to do for all condition, can I generalize it for example, I am looking at say wound healing patch, I test it out on some animals can I un use the same knowledge say for example, for burn wounds can I use the same knowledge if it is a (Refer Time: 18:33) wound it is a infected wound and so on. So, that is called generaliziability of the results.

Ease and adaptability to experimental manipulation, so, I am going to do lot of experiments on the animal. So, are they animal adaptable to the conditions is it easy for them to acclimatize ecological consideration. So, what are the ecological factors that coming to ethical implications what are the ethical implications involved what is a ethics using so many animals and so on actually so, all this need to be considered when we selecting an animal for our preclinical study.

(Refer Slide Time: 19:13)

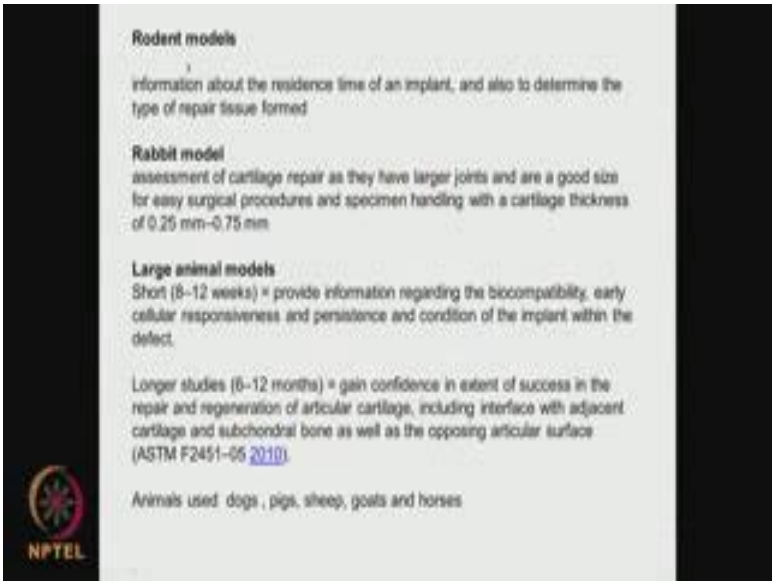


For example, if I am doing cartilage repair cartilage you all know this defects cause morbidity for patients and it becomes very difficult for surgeons trying to repair cartilage sports men get this old people get this especially those who have lot of physical activity get this. So, nowadays lot of thought is being given on use of biomaterial for repairing this why it is so difficult because a vascular nature of the chondral surface and the specialized rigid extra cellular matrix with a low cell density.

So, this tissue rarely regenerates by itself this tissue does not because a got a low cell density it got a specialized rigid extra cellular matrix a vascular nature it not a vascular system. So, what are currently they do try to do bone marrow stimulation cartilage plug transplant and expanded autologous, chondrocyte implantation. So, different types of; that means, they try to implant cartilage they are trying to do bone marrow stimulation and so on that with little success. So, when biomaterials are being looked at using artificial cartilages we to do animal studies.

So, we need plan based on the size of the joint the cartilage thickness the depth and critical size of the defect which will not heal spontaneously without any intervention if there is a small defect it may heals spontaneously if it is a large defect it will not heal the age of the skeletal maturity; that means, generally this type of cartilage joint replacement or material study better results in young patient as against old patient load distribution of the stifle affordability and ease of animal handling which type of animal is we want to if I am going to try it out on horse it may be very difficult to do that sort of studies and there is lot of issues involved because of the size of the animal rodent models are easy to use the information about the residence time of an implant and also determine the type of repair tissue formed what are the tissues that are formed. So, it gives you some knowledge.

(Refer Slide Time: 21:29)



So, you may try to do a rodent model initially then you may go into rabbit model looking at cartilage repair as they have larger joints when compare to rodent whenever a good size for easy surgical procedures specimen handling. So, we can look at cartilages of this thickness 0.25 to 0.75 go to large animals like dogs pigs sheep you can do short studies long studies 8 to 12 weeks this provides information regarding the biocompatibility cellular response persistence and condition of the implant within the defect.

Longer studies we can gain confidence in the extent of success in the repair regeneration of the articular cartilage including interface with adjacent cartilage and sub chondrol bone as well as they opposing articular surface this is the ASTM standard which on which this cartilage repairs or carried out using by different types of biomaterial. So, if you are talking about large animals dogs pig sheep goats and horses are used. So, one can look at short duration one can look at longer study.

(Refer Slide Time: 22:47)

So, what are the advantage disadvantages of various in vivo models commonly used in the assessment of biomaterial strategies for cartilage defect this is obtained from this particular reference mouse low cost manageable easily available transgenic available; that means, we can create different transgenic spices of mouse if you want to study can be used in subcutaneous and intramuscular, but then the joints are very small. So, joints in situ examination is impossible rats low cost easily available you can maintain in house permanently open growth plates accelerating intrinsic healing increased density of cells

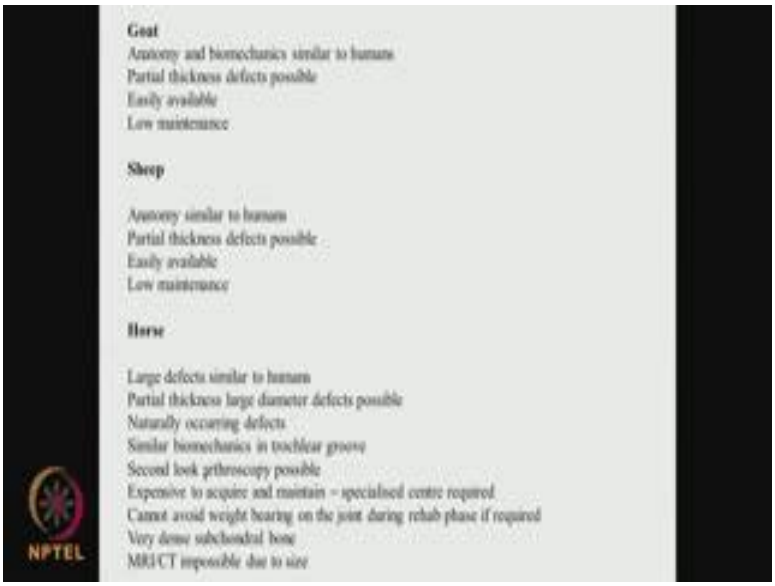
in cartilage causing more efficient healing, but if you want to partial thickness defects then they are not good.

Rabbit low cost maintain in house increased intrinsic healing due to increased cell density very different load characteristics consistent partial thickness defects, but very difficult to achieve. So, you see good advantages, but disadvantages good advantages disadvantages good advantages. So, if you are talking about small animal models for looking at cartilage then hmm you are having certain advantages and disadvantages and selecting the correct animal is a big challenge.

(Refer Slide Time: 24:08)

Again, if you are looking at large ones like dogs and pigs naturally occurring disease state because dogs also get this type of tendon issues cartilage just like human beings. So, they do corporate thin cartilage small critical size defects. So, we can study very small, but then the ethical issues are very very problem array pig biochemistry similar to humans bone apposition rate and trabecular thickness similar to human. So, we can study very nice partial thickness defects its expensive difficult to obtain at skeletal maturity specialized habitat then it is very special habitat there very also temperament.

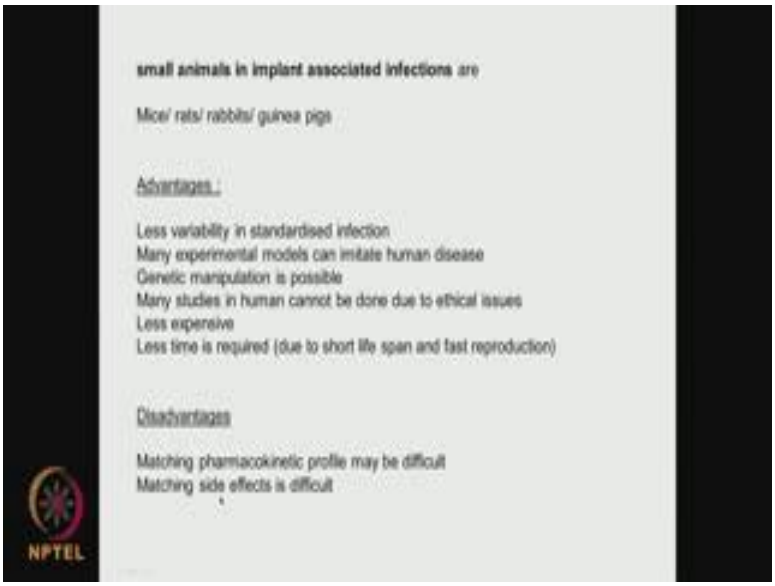
(Refer Slide Time: 24:55)



Look at goat anatomy and biomechanics similar to human's partial thickness defects possible easily available maintenance is also very low.

Sheep, again maintenance very low anatomy similar to human's partial thickness defects possible easily available look at horse. So, large defects similar to humans partial thickness large diameter defects possible naturally occurring defects similar biomechanics in trochlear groove second look arthroscopy possible, but it is very expensive to acquire and maintain we need very specialized centers cannot avoid weight bearing on the joint during rehab phase very dense subchondral bone MRI, CT all very very difficult because they are huge. So, you see small animals large animals many of them have advantages disadvantages. So, there is a big challenge especially when we do this type of preclinical studies before they really venture into human volunteers.

(Refer Slide Time: 25:55)



So, next one is implanted associated infections as I one mentioned long time back biofilm formation on implants and implant associated infection is a very serious problem as soon as a biomaterial is placed inside the body and. So, you need to study this again in small animals. So, one can create this type of a infection on certain implant surface biofilm surface infection and place it inside the models and see the animals models develop these studies are extremely important and because as I said implant associated infections are a serious problem on the first 1 or 2 days and most of the biomaterials 60-70 percent of biomaterials fail because of implant associated infection.

So, mice, rats, rabbits, guinea pigs are very good models to study that. So, advantages less variability in standardized infection. So, we can create the same infection again and again test it on many of this animals many experimental models can be imitate human disease. So, we can create there are genetic models of mice which can be created certain types of a defect on (Refer Time: 27:09) gene to simulate human volunteers.

So, genetic manipulation is possible many studies is in human cannot be done because of ethical issues, whereas they can be done on say mice rats and rabbits actually they are also of course, less expensive less time is required because of short life span and fast reproduction. So, if I am looking at what is the effect of infected biomaterial on the reproductively or their future developmental stages we can study easily on these type of

animals because they have short life span and fast reproduction what are the disadvantages matching pharmacokinetic profile may be difficult.

So, I am looking at exact elimination rates exact degradation rate of biomaterial it may be very difficult to match with the human volunteers matching side effects is difficult. So, the side effects these animals may get and side effects the human volunteers who may get may not exactly match. So, that is a very serious issue these are called disadvantages on animal studies. So, we will continue more on this animal studies use of animals and biomaterials research in the next class as well.

Thank you very much for your time.