Biomaterials for Bone Tissue Engineering Applications
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Module 5
Lecture No 25

So we can continue our discussion on the safety testing of medical device.

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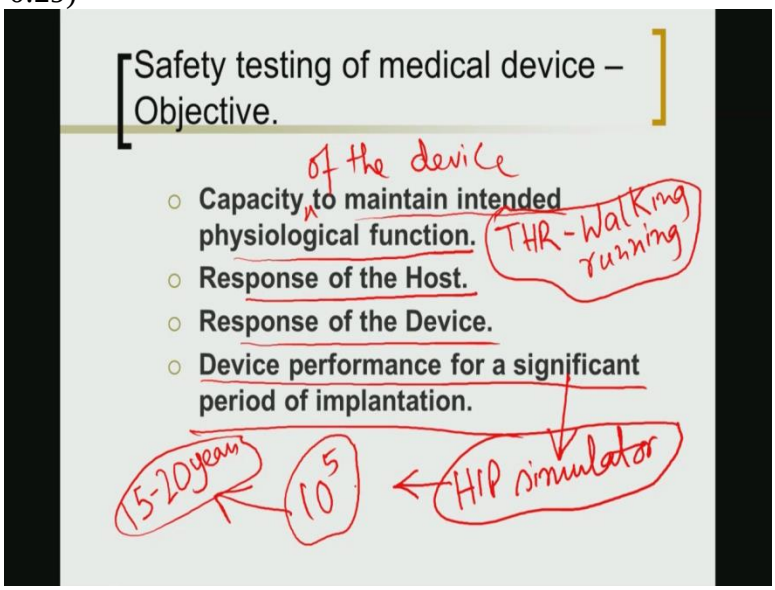

The objectives of this kind of testing is that, is that first to assess whether the intended physiological function, whether this device that which you fabricated. So essentially to make this phase complete the capacity of the device to maintain intended physiological function.

Now intended physiological function, let us take the example of THR, so the intended physiological function would be walking, or in case of very younger patents just little bit running. So whether this kind of physiological functions are compromised after a patient receives this total hip joint replacement that needs to be assessed. Second one that what is the response of the host and third one what is the response of the device itself and fourth one is that whether this device can perform for a significant period of time.

So essentially one can do some performance, device performance testing for the same example that I just mentioned total hip joint replacement, one can use the hip simulated experiments. Now there one can use 10 to the power of 5, 10 to the power of 6 number of fatigue cycles and from

there one can see whether this material can sustain without failure. This is such a large number of fatigue cycles.

And on the basis of such performance one can further predict this material can survive in human patient maybe for the time period of 15 to 20 years. But this is just a prediction, but when you are using 10 to the power of 5 number of cycles, you are not actually experimenting the material under biomechanical fatigue conditions for such a longer time period.

But instead you are doing what we call some accelerated testing in the laboratory. Now so you predictions may succeed or your prediction can fail in actual conditions when a patient receives the same biomedical device and then they maintain certain regular activities, physiological activity and there it can so happen that the device can perform only up to, in respect to in a, in an appropriate manner for up to only 10 years.

However this kind of accelerated testing of hip simulator or different joint simulator testing is helpful at least to predict what is the total number of what is the life time or what will be the longevity of a new device that has come out of a specific research programme. So I repeat that the device performance testing is quite unique and they should not be confused or compromised with the simple lab scale testing of, lab scale testing of a coupon sample using standard area of material characterisation based techniques.

So for example hip simulator simulates the mode, fatigue type of conditions under the physiologically relevant loading conditions and then it keeps certain gate cycles. let us say certain fatigue cycles, and there, and there on the simulator machine it can be seen that whether this material can sustain what is the maximum number of fatigue cycles and on the basis of that this longevity of a device can be predicted.

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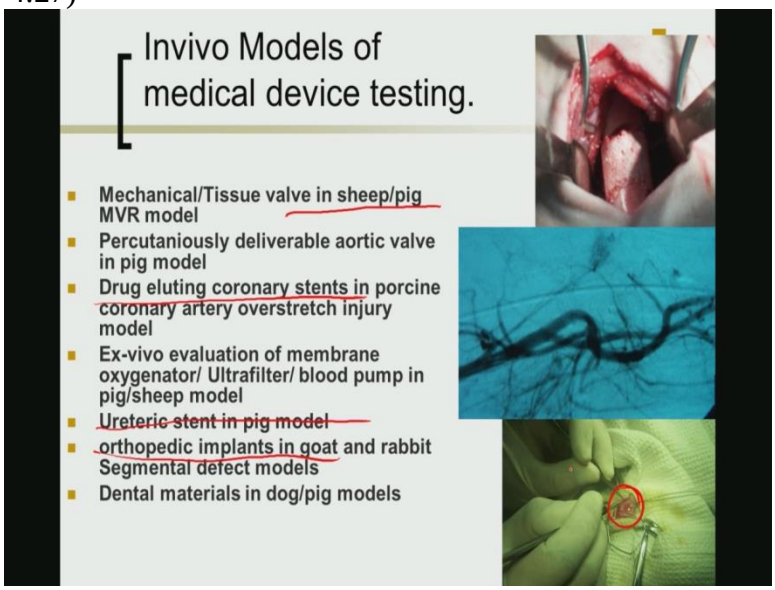

These are some of the examples of the different kind of pre-clinical trials one can do. For example mechanical or heart tissue valve can be tested in sheep or pig model that I have mentioned before. There will be drug eluting coronary stents in can be tested in a different animal model, then ureteric stent in a pig model can be tested, orthopaedic implants can be tested in goat model, dog model using that segmental defect model, rabbit segmental defect models or typical fumoral defects model. Dental materials can be used in dog or pig models.

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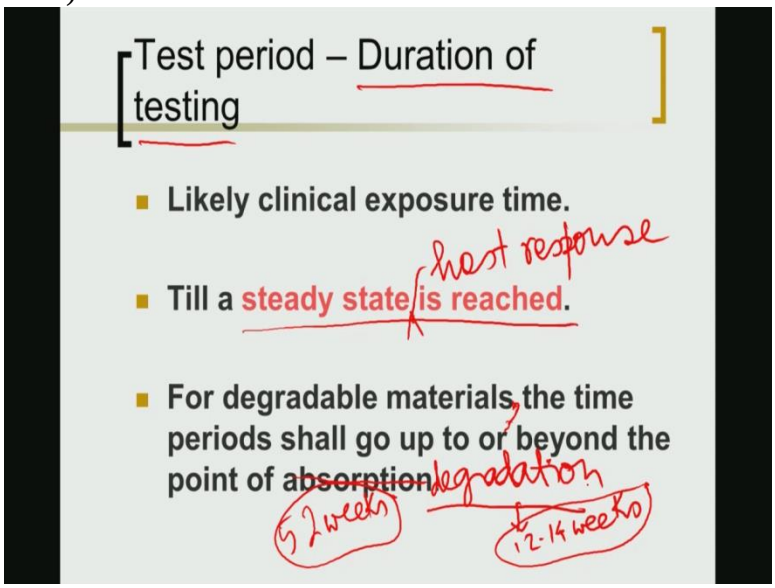

Ok, now some of the questions that one needs to address while designing this kind of pre-clinical study is that what would be the duration of testing. Then duration of testing should be, should have some similarities with the likely clinical exposure time like, or it has some co- relation with the likely exposure time. Another way that one can also fix that duration, testing duration is to see that at what time point the steady state, host response is reached.

That means that either fibrous encapsulation or certain inflammatory cell activity is reached a steady state and that is the time frame that one should use in actual pre-clinical study. But for degradable materials in one of the examples I have shown in the last module, the material completely gets off after implanting in an animal model living just a residue of that material.

So this is the sign of a large scale degradation in a physiological medium, physiological environment. Now for those degradable materials, one has to fix the time period of implantation beyond the point of complete degradation. Ok, so this is the, beyond of complete degradation. So let us say if the degradation takes place over 12 weeks or 14 weeks, so if these degradation takes place 12 to 14 weeks then one should do this pre-clinical study for let us say 52 weeks or so on.

Why such a longer time period is required? Because once you do this (degrade) once you know that this material degrades in pre-clinical, in any animal model, in three months, then subsequent 3-4 months, you need to study that how this degraded, degraded product is now distributed biologically in the total body and how it causes systemic or local toxicity to the tissue in different vital organs.

So the degradation products is not stationary or is not located at any given place but it is expected that the degradation products will be bio distributed and to cause some toxic effect to the different vital organs.

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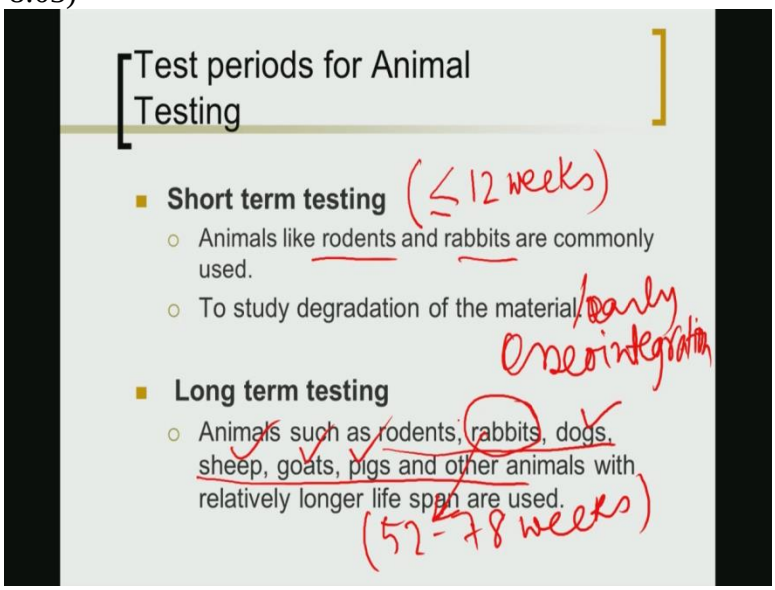

Now in this context there are two types of testing and according to the ISO standards that any testing which is less than 12 weeks of testing we call it is a short term testing and this is animals like rodents or rabbits are commonly used and to study degradation or osseointegration of the material or early osseointegration of the material. The long term testing which is typically somewhere around 52 to 78 weeks, this is the time span, this long term testing is done.

This is conducted with little bit larger animals so that it has a larger time span a lifespan, for example dogs, sheep, goats, rabbits, pigs and other animals. Often rodents and rabbits it may be difficult to maintain those experimental animals for sufficiently longer time period of 52 to 78 weeks. Therefore the larger animals like dogs, sheep, goat, or pigs are used for long term testing as I said with relatively longer life span.

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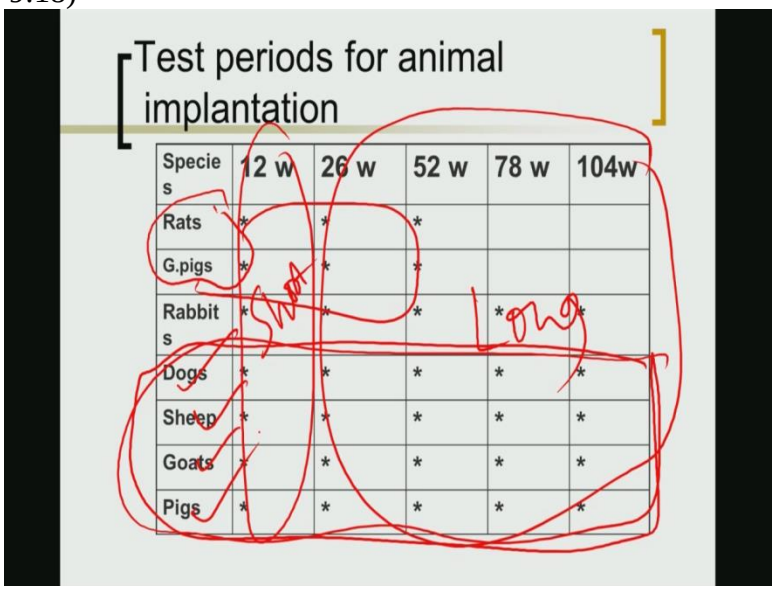

This is a kind of summery table like which are the animals, which can be preferred for short term and long term. So this is your short term implantation and these ones are your long term implantation. So here you know since rats, guinea pigs these are all like short term cases and little bit up to 58 weeks, but certainly this many times people have seen, certainly rats or mouse they cannot survive such a longer time period once the implantation, once you start doing this implantation experiments.

And this, all the four animals which are mentioned towards the end of this table or towards the latter half, like dogs, sheep goats and then pigs they are largely used for this long term implantation.

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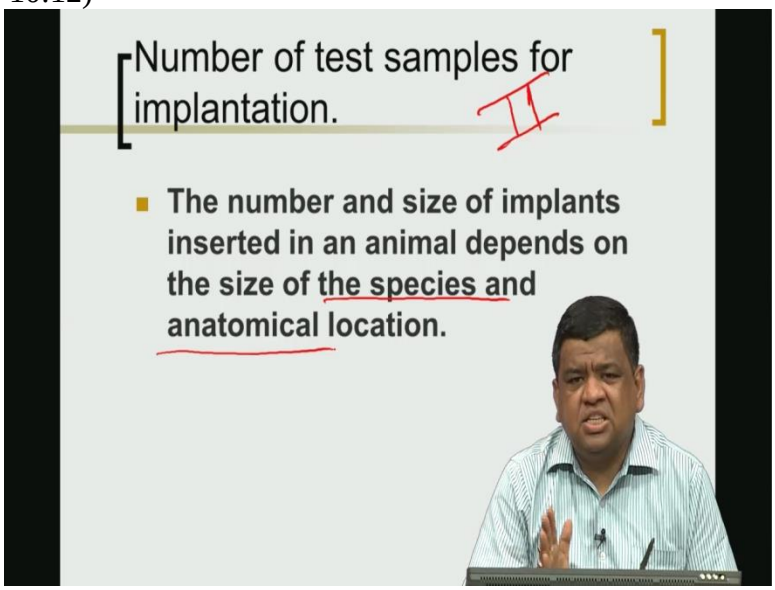

The second consideration, so first consideration as I said this is the consideration number one, the test period of the animal testing. The second consideration is that what is the number of test samples for implantation and the number of size of the implants inserted in animals depends on the size of the specious and anatomical location. So it also depends on the size of the experimental animals we are using. For example if you use mouse or rat as your model, animal model, then certainly you cannot use large material samples or large biomedical device samples in those animals.

So you need to use miniature size or smaller scaled down size of the material device or material devise in those things. And also anatomical location whether it is subcutaneous region like under the skin you are putting this material or you have to open the heart and you do this open heart surgery in case of the pig model and then put certain artificial heart valve or certain cardio vascular patches in those things. So it all depends on what this anatomical location and size that you are using.

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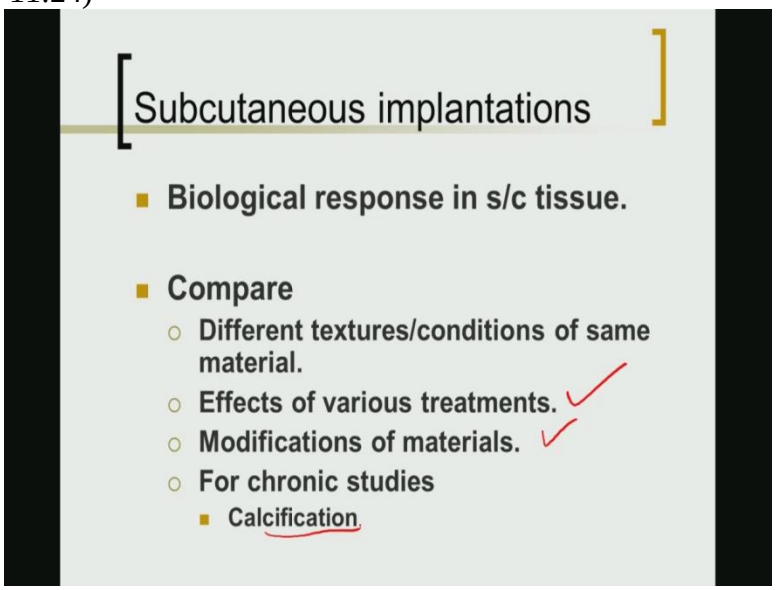

So just now I mentioned subcutaneous implantation. Here one can see that, what is the effect of various treatment or modification of the materials or for chronic studies like influence of calcification.

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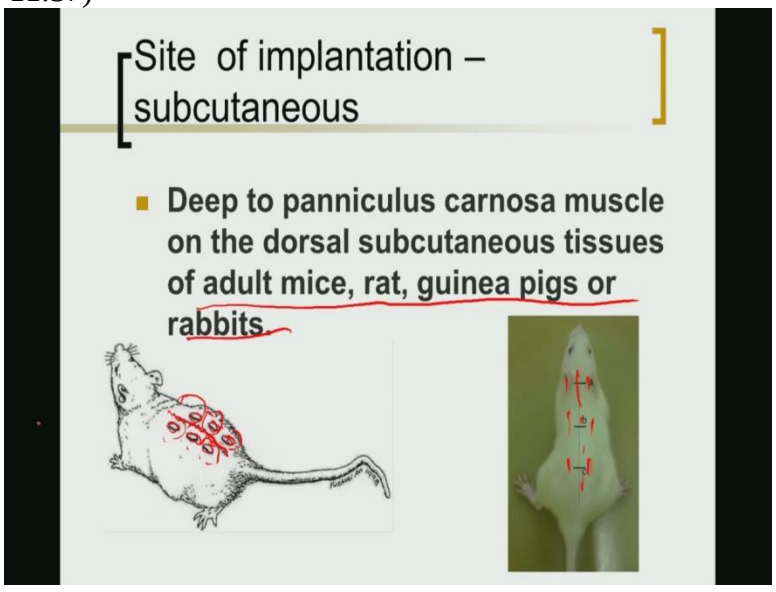

So this is the typical example of the subcutaneous implantation which can be carried out in adult mice rat, guinea pigs or rabbits. You can see that this is the skin is now stitched but before that when the skin is 3, 3 6 materials are essentially implanted in the subcutaneous region in the two sides of the dorsal sides and you can see these are like marked here and this marked region can

be radiographed after it is implanted and then it should be, it should be radiographed or X ray radiographs must be taken at certain regular intervals to see whether there is this local region around the implantation what I am trying to trace it with dotted lines whether there is, what kind of, what is the extent of inflammation after the implantation that one can see in this x ray radiographs.

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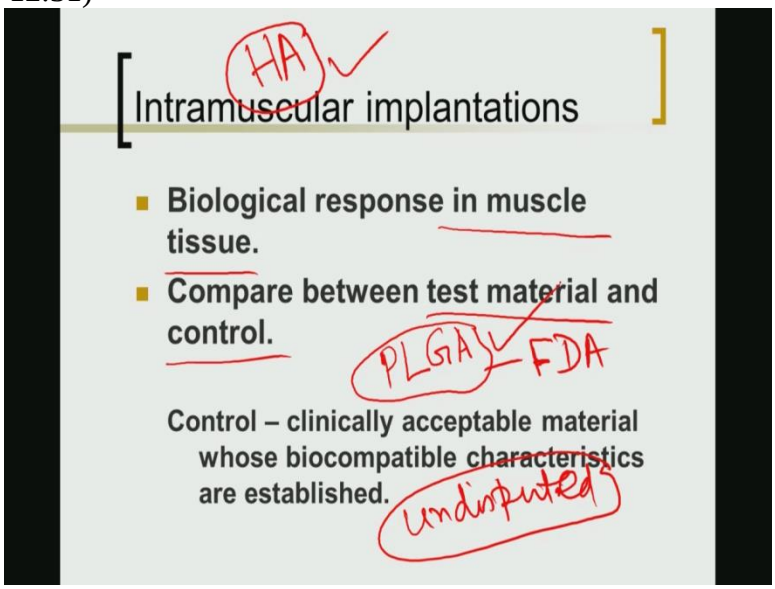

Now intra-muscular implantation, that is another type of implantation and there essentially one can see, what is the biological response in muscle tissues and compare between the test material implant and the control. Now control in case of this pre-clinical study, one can, one has to use clinically acceptable materials with with with proven biocompatible characteristics. So any material which is either clinically used in existing scenario or with, which has undisputed, let me put it this w, which has undisputed biocompatibility property or proven biocompatibility property in vivo.

So this many of this, I will just give you some of the examples. When people want to use that the efficacy of a new biodegradable polymer composition, they often use Poly lactic poly glycolic acid as one of the control samples because this PLGA, Poly lactic-co- polymer, poly lactic and ploy glycolic acid is and FDA approved material.

So it is a US FDA approved material, so nobody questions the biocompatibility of that material. So therefore this can be used as a control material. When people use different bio ceramic material then they use hydroxyapatite is another control material because hydroxyapatite is widely accepted as a most bio compatible inorganic material. So I have given you one example from the ceramic community, I have given you one example from the polymer community just to show you that how to select a control for your clinical study.

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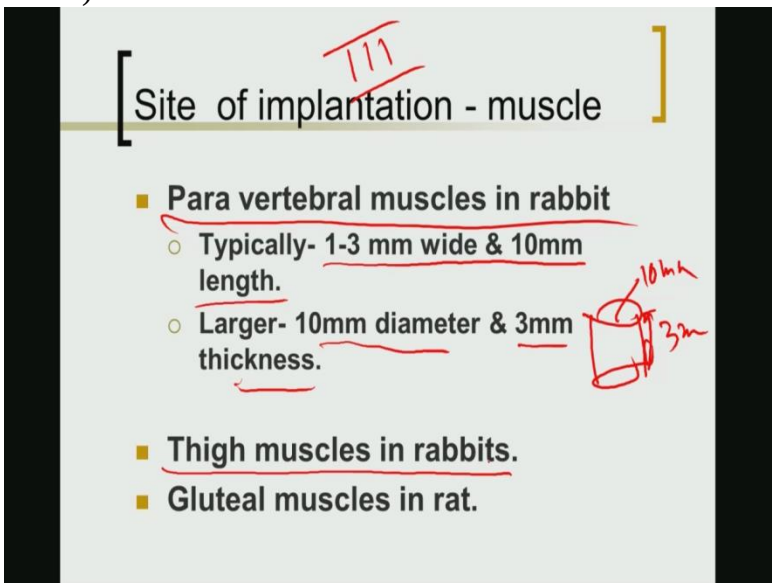

Third consideration is that what is the site of implantation. Whether it is a muscle, if it is a muscle then typically 1-3 millimetre width or 10 millimetre length sample should be used and sometimes a larger samples of 10 mm diameter or 3 mm thickness that is like cylindrical samples 10 mm diameter and 3 mm thickness also can be used just to put vertebral muscles in the rabbit. And this where one can put in the rabbit, is like thigh muscles or gluteal muscles in rats.

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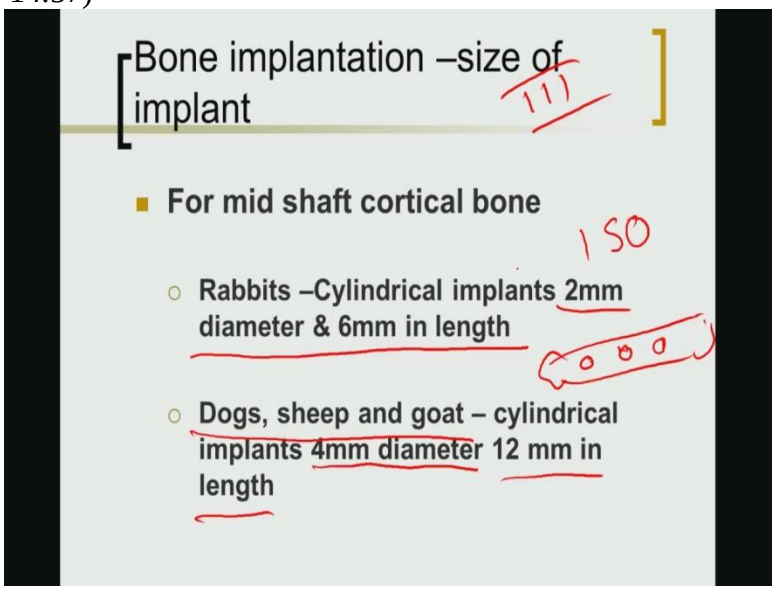

Fourth point is that, so, oh this is the third point, I am continuing that size of the implantation, in case of the bone related application like in case of rabbits, this is the kind of a standard guideline people use as per the ISO standard like 2 mm diameter and 6 mm in length and that is typically done in the femur. So if you have a femur then one can put the hole using the microtome and this, after making this hole then you can insert this 2 mm diameter sample.

In dogs, sheep and goats since they are little bit larger anima model so therefore larger sample size can be used or larger sample size can be tolerated during the pre-clinical experiments. Just to give an example on the numbers like in case of this dog, sheep and goat 4 mm diameter sample with 12 mm in length that means larger the size of this samples which can be implanted in rabbits , those kind of implants can be put it, in large animal model.

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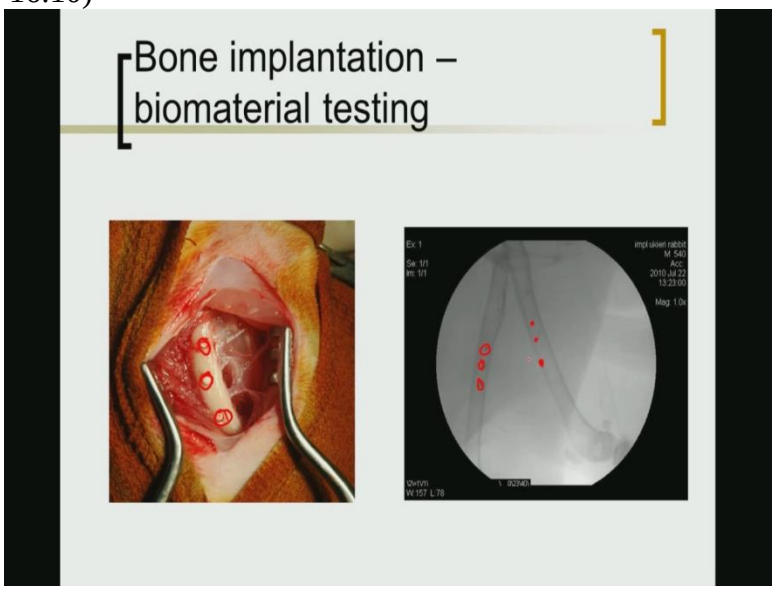

This is just an example that in the femoral, these three implantation sites, 1,2,3 so first this wholes are created, defects are created and then you can see that some material are being implanted. This is the X-ray radiograph of these three implants. You can see this is the two side of the two femurs are showing because as far as possible according to the clinically approved guide lines, that pre-clinical study involving an experimental animal the control implant and test implant must be inserted in 3 numbers in the same animal on both sides, on both side of the femurs.

Or in other words, in one femur will receive three test implants another femur of the same animal must receive 3 control implants, so that one can essentially see the efficacy of the biocompatibility of the implants.

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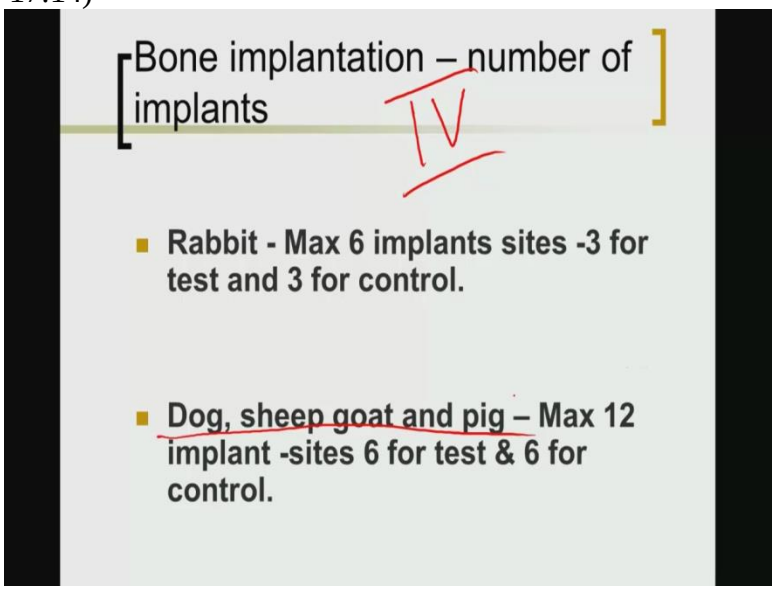

So what is the fourth consideration is that, what is the total number of implants that one needs to take in this particular test in case of rabbit maximum six implant sites as I mentioned 3 for test and 3 for control. In case of dog sheep, goat and pigs is the larger animals, so they have larger size of these bones, femur, so here 12 implants can be used like 6 for test and 6 for control.

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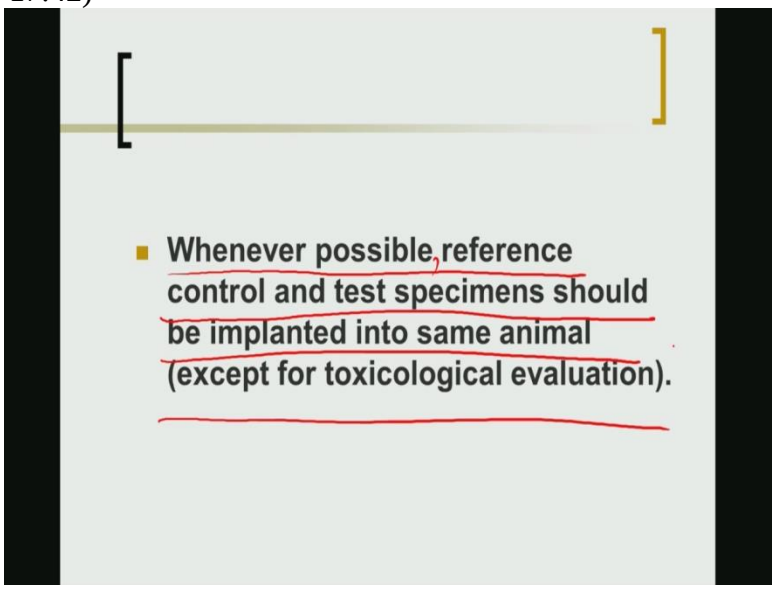

I think I have mentioned this point categorically and I have mentioned this in writing also, whenever possible refer this control and test implanted should be implanted in the same animal

except for toxicological evaluation. For all the osseointegration testing, for all the biocompatibility testing this should be done as far as possible.

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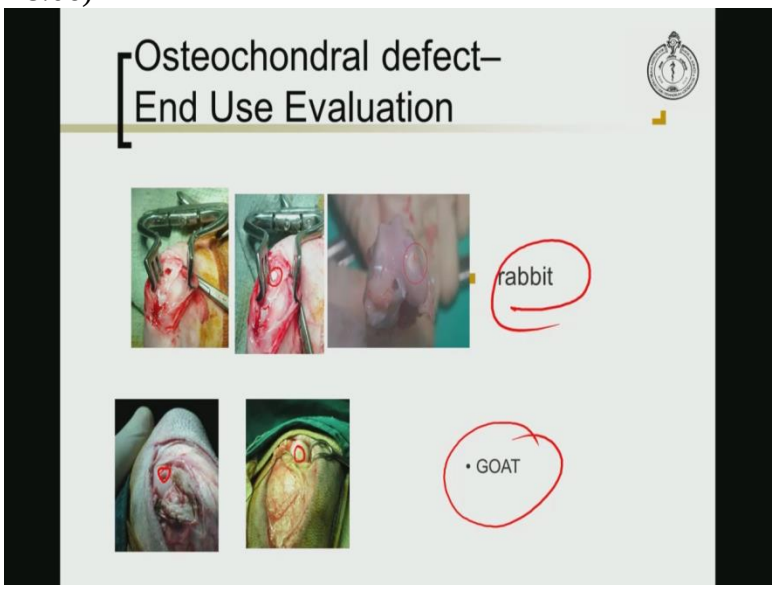

The other thing is that some of the example that is shown. For example osteochondral defect, you can see there is a small osteochondral defect and this is the defects field with the biomaterial and this is the examples in the goat animal model. This is the example of the rabbit animal model how this osteochondral defects, these are shown.

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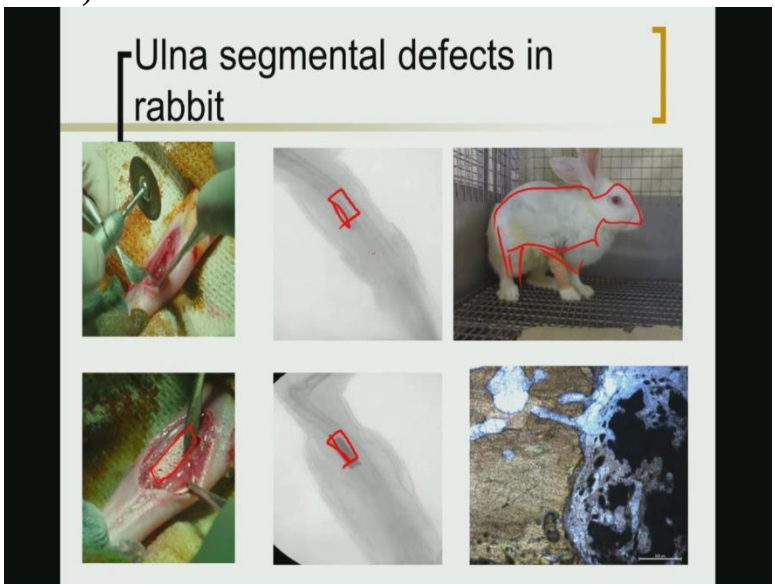

This is some other example that Ulna segmental defects in rabbit and here you can see this is a large porous block which is put in the ulna part of the rabbit. And this is the experimental rabbit which was used in this kind of experiments and this is the x ray radiograph of the implants after the implantation, after they are put into the ulna.

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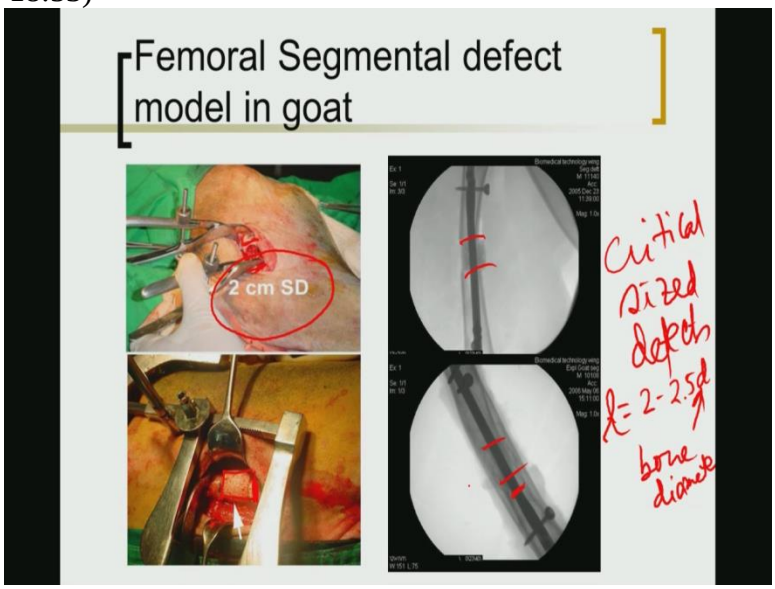

Segmental defects is one of the other things so there is something called critical size defects. So segmental defects or critical sized defects means so, when you cut through osteotomy a large region of the relatively large region of a bone, and then you insert that large, when you insert an implant which is of the equallent size into the bone defects osteotomy defect in that particular place.

Here it is shown that 20 mm length so that is a large defect is created segmental defect is created. And then you are filling that segmental defect with a biomaterial and this is what is being shown in the X ray radiograph also that is how this large segmental defect is being shown. Now typically if the if the defect length is 2 to 2.5 times the diameter of the bone, so these your bone diameter and the defect size the length is the your length created.

So if this is your bone that what I am showing. This is your bone small size bone, so in or this is that, in x ray you can see it more clearly. So what is the bone size, what is the diameter and approximately 2-3 times diameter or the length of the defect if you create in that animal then that

defect size can be called as a critical size defects. And this is one of the examples that has been shown in the goat model, that how this in the femur of the goat this large segmental defect is been created and this is subsequently filled by this porous blocks on the materials.

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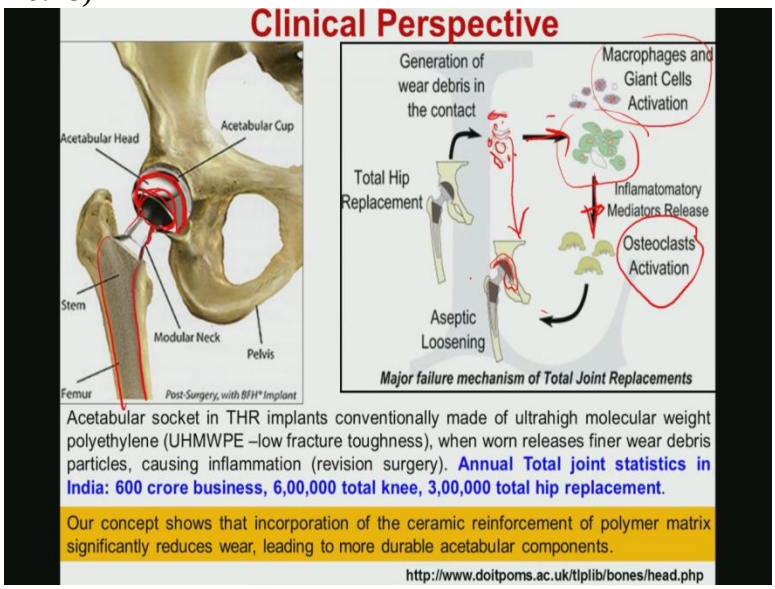

So before I conclude this pre-clinical study let me go through some 3-4 more slides. So apart from the general safety study, apart from this biocompatibility study apart from this overall osseointegration or osteoconduction aspect which is kind of hallmark of this in vivo biocompatibility experiments. Another important idea of conducting these in vivo experiments to assess the toxicity of the final debris articles. Just to give you some clinical relevance to the toxicity study this slide shows you that are the total integrated part of this total hip joint replacement.

This is a modular leg and this is your stent part which goes into the femur of that human patients and on the neck it is fitted to the acetabular fumoral ball head and which is actually in conformal contact with the acetabular socket or acetabular cup. Now during the walking, during running each time any regular activity with hip movement essentially involves the friction at the polymer, friction at the fumoral head and the acetabular socket interface and because of this friction you see this kind of smaller finer debris particles.

Now these are debri particles as you can see they are different in terms of both size and shape. And these debri particles, first of all they can cause inflammatory mediatory response and it can attract these macrophages and these giant cells, foreign body giant cells which you have seen. So foreign body giant cells are large multi nucleated cells which are activated towards the end phase of the fibrous capsule, fibrous tissue encapsulation and through this inflammatory mediator release they can activate the osteoblast cells.

Remember there are three types of cells I have mentioned in a typical bone structure osteoblast cells, which are essentially bone forming cells, osteoclast cells which are bone resorption cells and osteocyte cells, this is the mature bone cells or this is the aged member of the oestrogenic lineage during the differentiation of stem cells through the bones to the bone cells. Now at any given pint of time, is number of osteoclast cells is more or osteoclasts are more activated that means the bone will undergo resorption.

So there should be healthy balance between the osteoblast cells and the osteoclast cells. So at any given adult matured bone there should be more proportion of osteoblast cells compared to osteoclast cells, but in case of this wear debri induced biological effect that triggers the osteoblast activation and once it triggers then what happens? There is aseptic loosening effect takes place at the total hip joining replacement and the host tissue interface.

And these actually necessitates that any martials that you are proposing any new materials that you are proposing for total hip joint replacement must undergo toxicity analysis not in the bulk form but in its particulated form that means this materials must be present in a very small particulate shape and size. Mostly in the micrometre range so that their potential toxicity in any in vivo system or any biological system in any experimental animal can be appropriately assessed.

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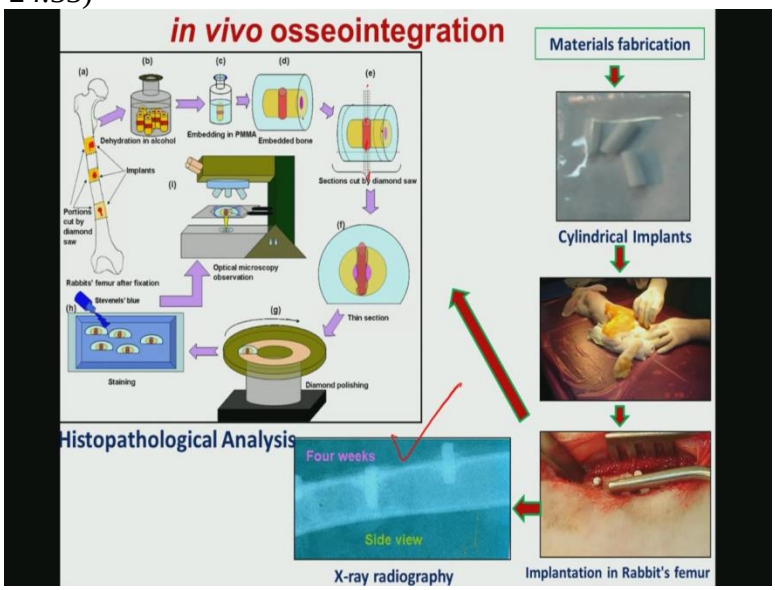

So this is the typical example of this histopathology analysis followed by in vivo osseointegration. I think I have mentioned these things also in the last to last 2 to 3 models back, that you have a fumoral defects here. So this fumoral defects, the implants can be put it inside a femur. Then after the implantation is over you can do a series of chemical analysis and sample preparation for the histology and which involves that you have to put this bone bone samples into certain resins.

Then you have to cut it through diamond saw or precision microtome then you can take a thin section, polish it. You have to use different staining agents, depending on what kind of tissue and cells that you want to identify in histology section and subsequently it should be done by the simple optical microscope. Other things you use quite widely that is X- ray radiography in this in vivo osseointegration.

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Now coming to the toxicity analysis of the wear debri particles. So this is the classical example of the let us say total hip similar experiments, so you do this kind of fumoral head and acetabular socket for example you can you this ceramic fumoral head and polymeric acetabular socket, you do a large number of cycles so that large number of wear debri particles of different size and shape are produced, you make it, you dissolve then in (poly) phosphate-buffered saline or PBS solution and so that you make the eluate of this particles.

Then after that you put that, inject this particles in the intra-articular region, as, as it has been shown here. The intra-articular region of the knee joints in the mouse model for example and then you let the mouse survive after getting this injection. Now after that, then you can sacrifice the mouse after a pre-determined period of implantation time for example two weeks, 4 weeks, 12 weeks or so on.

Then different vital organs like liver, kidney, spleen, lung, heart, their sections needs to be taken and that should be assessed in, using the standard histology sample preparation, that should be assessed for different different analysis to see that how this tissue is responded to this eluates or whether the eluates have caused any tissue level toxicity in those vital organs. Apart from this histological analysis one can do haematology and serum biochemical analysis so that any blood parameter is significantly changed or not.

Then subsequently analysis of enzymatic activities in synovium tissue needs to be done and fourth one is the pro-inflammatory cytokine expression analysis just to quantify what is the level of inflammation, inflammatory response. And I had mentioned that different types of experiments can be done for 4, 8, 12 weeks in typical mouse models. So these things are already reported from our group as well as various other groups in the literature. So I think here I end for the in vivo biocompatibility testing and I will start with a new model in the next time.