Biomaterials for Bone Tissue Engineering Applications
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Module 5
Lecture No 20

Ok, So we will continue our discussion on in vivo biocompatibility that is the preclinical studies on biomaterials, we we have discussed in the last module, that what are the biological events that take place at the implant tissue interface.

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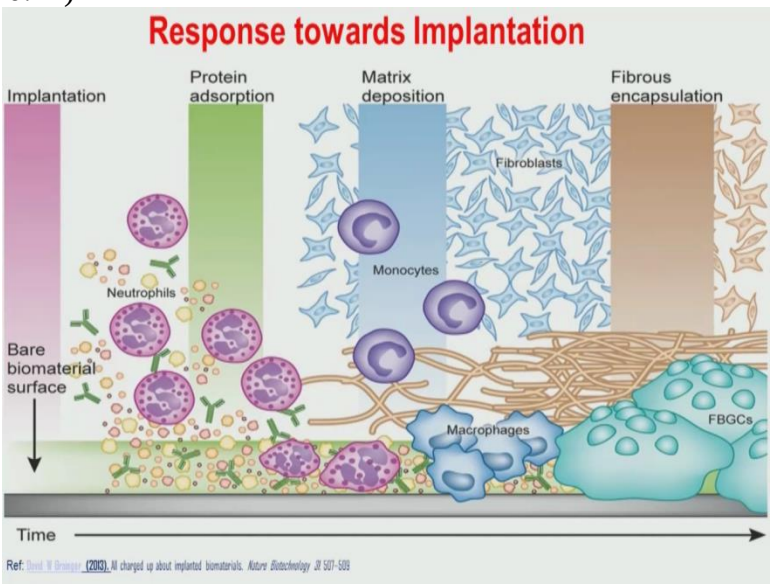

Particularly this slide I have spent significant amount of time to make you understand that this interaction first starts with this attraction of neutrophils towards this implant, synthetic implants, followed by similar attractions for fibrinogens protein adsorption. This acts as a precursor for the adhesion and functionality of the fibroblast on the protein (()) (1:14). Simultaneously the other cells like monocytes also adhere along with this fibroblast.

Now once this large number of fibroblast they are functional on the material substrate interface, then they will secrete collagen and then it will form a collagenous matrix of the extracellular collagen, collagen based extra cellular matrix which will ultimately shield the biomaterial through the formation of the fibrous encapsulation. And along this time scale, just before the fibrous encapsulation is matured then foreign body giant cells also, then foreign body giant cells

activity also becomes prominent as well as the macro phases. So this entire thing takes place over a period of 3-4 weeks so this is the time of post implantation.

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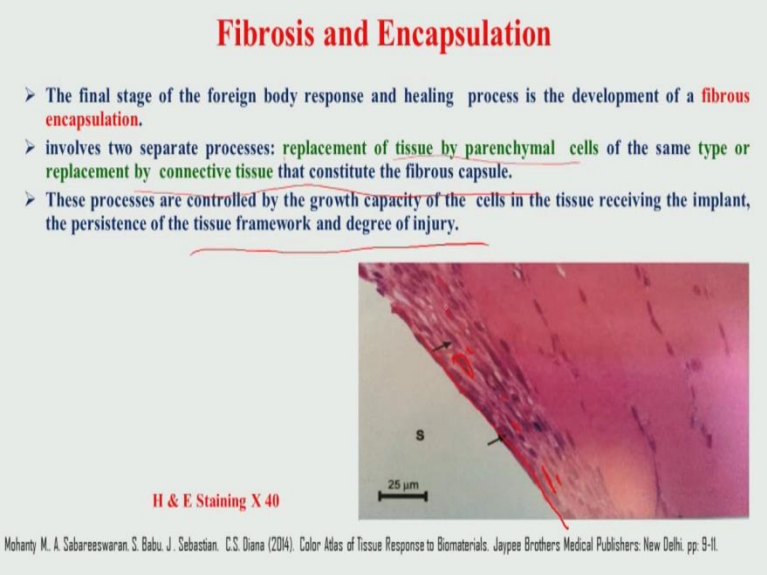

Now what I will do in the first half of this module, I will just try to finish the scientific discussion and with certain examples taken from the literature just to make you understand that how this in vivo response can be characterised in the context of the biomaterial, in the context of the orthopaedic biomaterials particularly. So essentially this fibrosis and encapsulation involves two separate processes.

One is the replacement of tissue, by parenchymal cells of the same type and second one is the replacement by connective tissue that constitutes the fibrous capsule as you have seen before. And this process sincerely are controlled by cell growth capacity in the tissue receiving the implants and the persistence of the tissue framework and degree of injury. So these things have been mentioned very clearly in the last slide also.

Now other things you can see here. This is the histology slides and this part of the slide is essentially haematoxylin and eosin stained region where you can see a lot of cellular activity at the interface of the biomaterials and the tissue.

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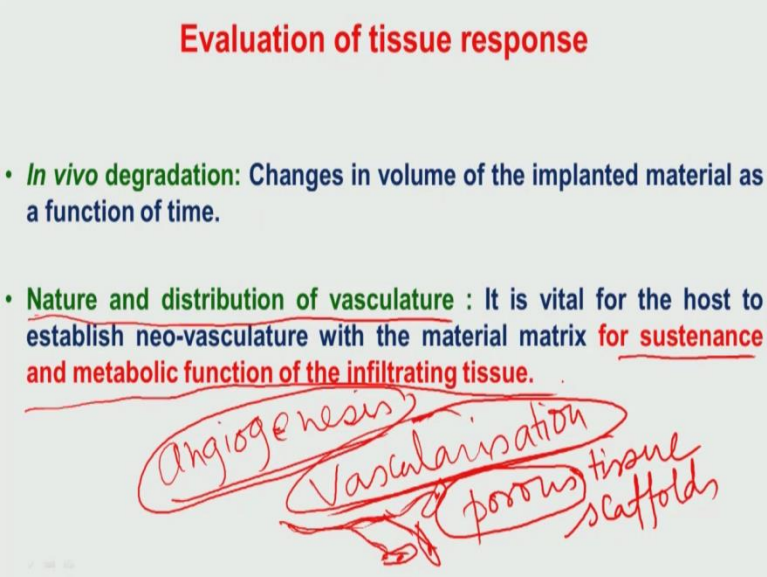

Now two ways you can evaluate the tissue response, one is the in vivo degradation that can be quantified using volume changes or changes in volume of the implant and material as a function of time. The second one is a nature and distribution of vasculature and here I may like to recall you the two concepts that I have mentioned in the last module. One is angiogenesis and second one is the vascularisation. These two these two concepts were defined in the last module. They appear to be synonymous and this is particularly more relevant, this both the concept for the porous tissue scaffolds.

Essentially they are quantified by new blood vessels, that are formed into the porosity of this 3 dimensional interconnected porous scaffolds and so this, how this blood vessels are growing inside and so that you know, this blood and oxygen can be, blood and oxygen can be transported to the cells as you can if you recall this kind of picture that I have shown, and at the same time how these blood vessels are branched out so that different, these blood vessels, they can penetrate more and more into the different tortuous porous path.

So these two things can be quantified by these angiogenesis and vascularisation. And many times in tissue engineering, people do use angiogenic growth factor just to stimulate this process of angiogenesis. So before implantation people use angiogenic growth factor and so that this angiogenesis can be promoted once this tissue engineering scaffold is implanted into the animal tissue. So that is what I have mentioned this is the nature and distribution of vasculature, also is

important and this also has relevance for sustenance and metabolic function of the infiltrating tissue.

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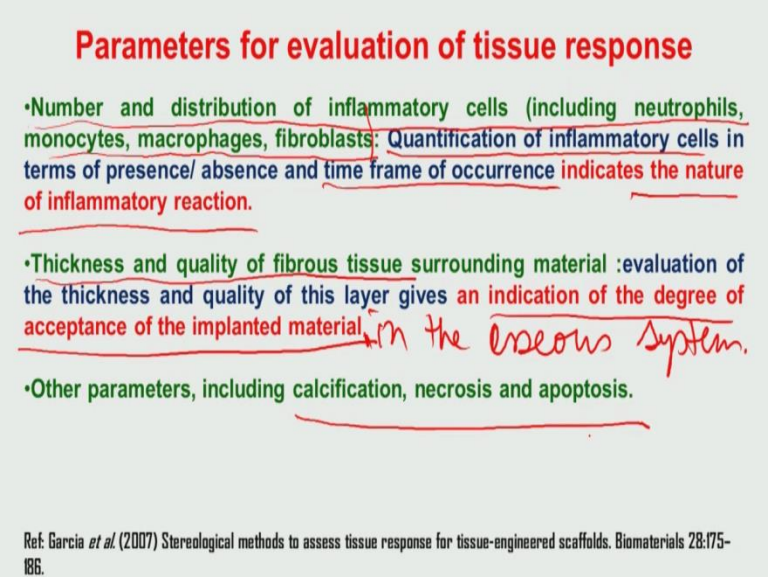

Now the, three more factors which are mentioned here. So all together there are multiple factors which influence this angiogenesis, multiple factors which influence the tissue response. Now among these two factors were mentioned in the last slide and this slide certainly mentions or summarises three more factors. Third one is the nature and distribution of inflammatory cells including neutrophils, monocytes, macrophages and fibroblast. Now this kind of quantific, this requires lot of quantification based on excessive analysis of a number of histology slides.

So essentially you can see that how this different kind of cells, which can be clearly marked based on the appropriate staining or based on the appropriate tissue or cell specific stained tissue specific staining agents and their quantification in terms of how this number of cells in terms of the presence or absence and the time frame of their occurrence, like at what kind of time frame, time scale after the post implantation that fibroblast keep on increasing or they decrease and how the changes takes place over time frame that indicates the nature of.

So all these cellular activities essentially constitute the inflammatory action. It is important to recall that irrespective of biomaterial composition, irrespective of the way a biomaterial is placed in any given experimental animal through surgery or by injection of this biomaterial (())(07:56)

inflammation cannot be avoided. Only the degree or the extend of inflammation can be modulated or can be reduced through certain through particular biomaterial, through the use of particular biomaterials.

So therefore inflammatory cells or presence or absence of different the other cell types as we mentioned neutrophils, monocytes etc., their quantification is indeed necessary and this quantification is more important so that one can develop both a qualitative and quantitative understanding of the tissue response upon implantation of an implantable biomaterial. Ok I hope I have made this point very clear.

The fourth point is the thickness and quality of the fibrous tissue. Now this thickness and quality of the fibrous tissue means that this actually indicates the degree of the acceptance of the implanted material in the osseous system. So essentially this thickness certainly would increase with time, this thickness of this fibrous tissue certainly many times goes linear or many times goes in a non linear manner with the time in implantation.

Now other parameters which one can also look for while analysing the histology slides is that, the signature of calcification, necrosis and apoptosis. So remember necrosis and apoptosis is the two ways that cell death can take place and which have been introduced or discussed quite a significant extend in some of the preceding modules.

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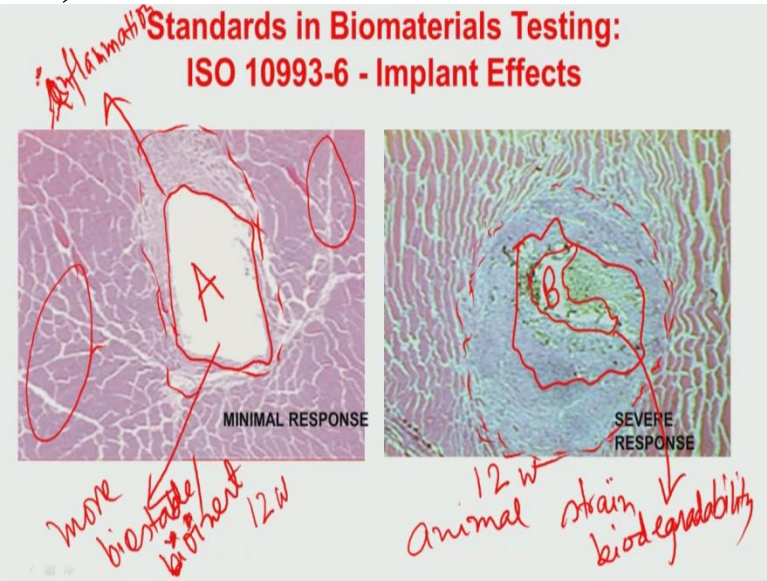

Now these are the two examples where a biomaterial where you can see this biomaterial has a little bit irregular shape. It was implanted in an animal and according to ISO 109936 implant effects you can see there is certain regions of the inflammation. So you can see that region of inflammation.

So this is the region of inflammation and sorry, this is the region of inflammation and certainly there the tissue structure or the appearance or morphology of tissue is certainly different from an area which is remote from the implant. Now side by side you can see that another material of similar shape or size but of different composition when they are implanted initially in this particular shape you hardly see any material left after the implantation for same material, for same time period.

For example if this is implanted for 12 week in the same material the other is implant for 12 weeks for different materials, so let is say this is material A, this is a material B, so you would like to see that which kind of material exhibits minimum response or severe response. Remember minimal response cannot be avoided. So whatever is the material composition I repeat there would be inflammatory response. Now, when you compare this kind of situations you have to make sure, that you are using same animal strain.

Like if you use New Zealand rabbit, New Zealand male rabbit so you have to use both the cases, New Zealand male rabbit. Second important thing is that, you have to also use similar kind of size and shape of the implant material in both the cases. Only thing you are allowed to change is the chemistry or chemical composition of A and chemical composition of B. So therefore you can compare now, what is the tissue response in case of A and what is the tissue response in case of B and certainly you can see in case of B, the material is almost gone.

So it leaves only certain traces of the material B and also if you se that larger area much larger area which I am tracing by dotted arrow, this larger area of the tissue is now inflamed or is is showing the signature of severe inflammatory response.So certainly, material B shows more biodegradable in nature. So it certainly shows reflection of biodegradability as well as severe response whereas this is more like biostable or bioinert kind of behaviour.

So from these tissue response also on the basis of tissue response you can essentially, you can essentially distinguish between two types of response in one case you see bioactive, biostable or bioinert response, another thing is biodegradable response. In one case left-hand side material shows minimal response whereas the right-hand side material severe response in the same animal strain.

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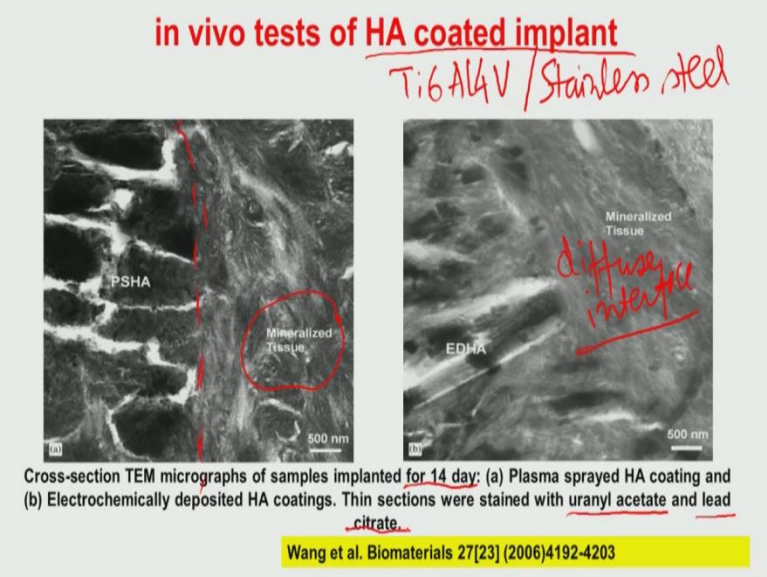

Ok, these are some other examples, which is shown for hydroxyapatite coating. Remember this hydroxyapatite coating is widely used on the major orthopaedic implant alloys, like titanium 6 aluminium 4 vanadium or 316 L stainless steel stem. So this hydroxyapatite coating depending on the way these coatings are deposited on the substrates whether it is called electrolytic position or whether it is called plasma spraying.

Plasma spraying is by the way commercially most widely used, electrochemical deposition as the name suggest it takes place more in the room temperature deposition condition as far as plasma sprain involves very high temperature deposition of hydroxyapatite on this titanium 6 percent aluminium 4 percent vanadium or stainless steel substrate. In both the cases they are implanted for 14 days in rabbit and animal strains and this was reported in the journal biomaterial almost 10 years ago.

Now what you see here that if you consider that the left one PSHA plasma sprayed hydroxyapatite coating. Very clearly you can see the evidence of these bone tissue intervals, sorry material tissue interface and also you see clear features of the mineralised tissue. Mineralised tissue means it has calcium phosphate content. Whereas in case of electrolytically deposited hydroxyapatite coating this similar evidence is also observed but here the interface is more diffused interface.

Here again the mineralised tissue forms but the interface is clearly more diffused interface and this is that these images were taken in the transmission microscope and by brightfeild imagining, and prior to the transmission microscope this tissue section were stained with a particular staining agent like uranyl acetate and lead citrate so that easier visualization is now possible and so that one can confirm the mineralised tissue formation.

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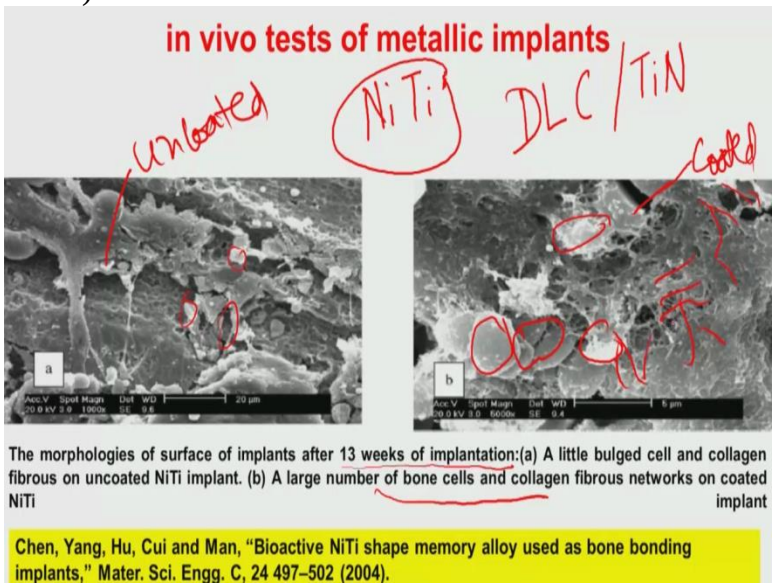

Some more examples, nickel titanium this is the classic case for the nickel titanium. Now what is the relevance of the nickel titanium? Nickel titanium is used for the cardiovascular applications of stent of cardiovascular application and people use certain coatings nowadays like ceramic coatings so that you know cardiovascular applications that hemocompatibility or blood compatibility is very important as I mentioned earlier, in one of the earlier modules.

And to improve the blood compatibility one has to use certain ceramic coatings and some of the coatings that I have mentioned earlier if you remember, one is the diamond like carbon coating or titanium nitrate coating. Now these diamonds like carbon coating or titanium nitrate coating are normally deposited on the titanium or nickel titanium substrates to improve their hemocompatibility property. So next level question, therefore arises whether these kind of coating deposition or nickel titanium will have some influence on the way tissue formation takes place or way tissue compatibility is influenced in the in vivo condition.

And for that again this implants were put it into rabbit animals for 13 weeks. Now on the left-hand side you can see certain bulge of these cells here like bone cells and collagen on the this is the uncoated one and this is the right-hand side this is the coated nickel titanium, this is the coated nickel titanium. And what you see on the right-hand side that you see that more number of bones, this is the osteoblast cells here and also this is a collagen fibrous network. This fibrous network actually, essentially signature of exocellular matrix deposition along with the bone cells on this particular substrate that is ceramic coated nickel titanium.

So in both the cases I have mentioned that if you put coatings, certainly this tissue response is, certainly tissue response is enhanced.

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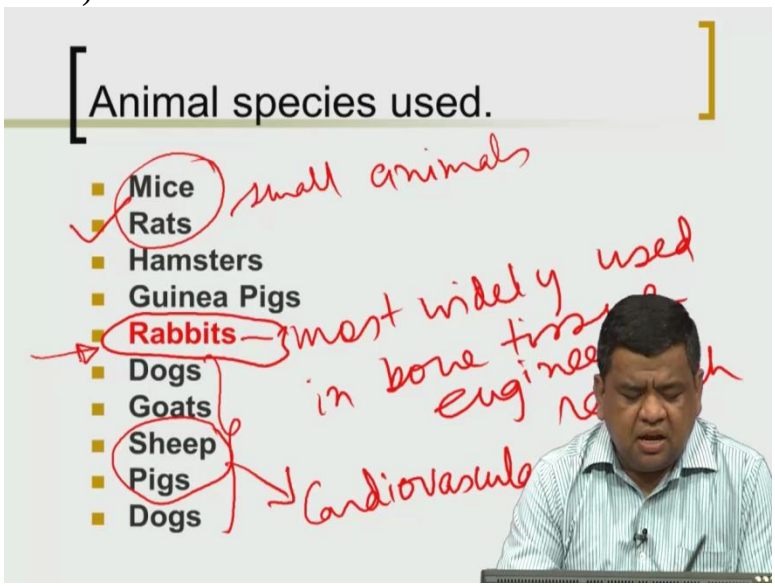

Ok now having given this background on the tissue response in terms of the scientific understanding, I will now explain, some of the important issues related to how to design this kind of pre-clinical study in terms of how to select animal models, which kind of animal models are to be selected for what kind of experiments and second thing is that what kind of defect model is to be used for particular implantation. This is a typical list of the animal models which are widely used in biomaterial research.

Now typically, so the first two, first two mice and rats, these are like small animals and why rabbits are written in different font colour, just to highlight the importance of rabbits or just to highlight the fact that rabbits by far most widely used in bone tissue engineering research. Since the name of the course is Biomaterial for bone tissue engineering applications, therefore I wanted to highlight that which particular animal strain which is used most extensively in bone tissue engineering applications.

The other relatively larger animal models is the dogs, Goats, sheep, pigs and out of that sheep and pig model is the larger size animals and they are more used for cardiovascular research particularly for the heart valve applications or any kind of design related issues in the heart value when one needs quick clinical study, then one has to use the sheep and pig model. Other things, I will also mention later or perhaps while summarising this pre-clinical study, is that even if some materials are tested for their pre-clinical efficacy in the small animal model, then ideally that material is biocompatibility is to be subsequently proven at still larger animal .

Or in other words simply carrying out experiments or conducting pre-clinical experiments in small animals is not sufficient enough to claim the in vivo biocompatibility of that particular animal or on the basis of small animal model experiments only clinical trials in human patient should not be conducted. So therefore one, therefore researchers are encouraged to conduct these experiments, to start with small animal models then validate the small animal model by biocompatibility result in a relatively larger animal or medium sized animal further, to further plan for the clinical trials.

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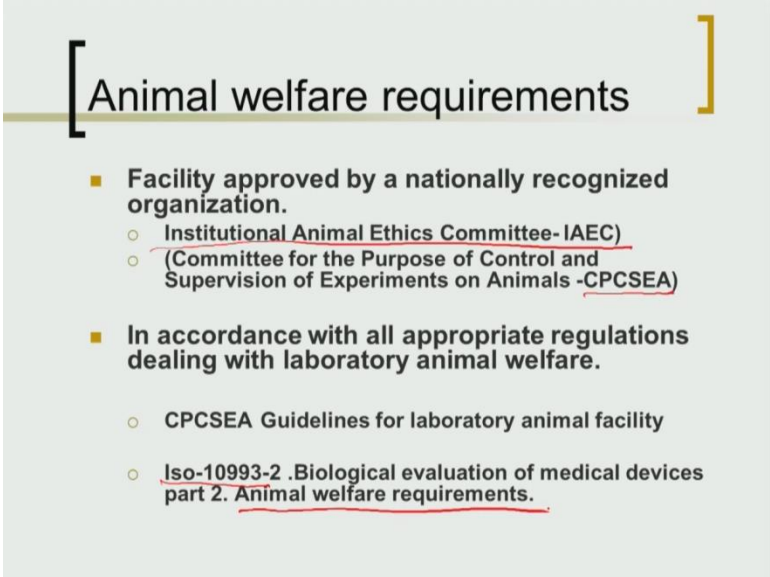

Ok, so animal welfare requirements, is one of the key things, basically now I will show you, how to kind of what are the perspectives on which animal pre-clinical study needs to be designed and what are the factors need to be used or need to be considered while designing these kind of animal experiments. So in terms of animal welfare requirements, I mean which is which varies, which essentially vary from country to country, first of all any animal testing facility must be approved by nationally recognised organisation and therefore one should have Institutional Ethical Committee in place.

Institutional Ethical Committee also should be approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals which is abbreviated as CPCSEA. Now this Animal Ethical Committee not only consists of veterinary surgeon who actually does this animal experiments but also consists of biologists, any lay man or lay person or common man from the society as well as a lawyer who can who knows the rules related to Animal Ethical Committee guidelines and so on.

Now in accordance with the appropriate regulations dealing with the laboratory animal welfare one has to subsequently follow the different guidelines like ISO Standards like ISO 109932, there is biological evaluation of medical devices that is part 2 of the animal welfare requirements.

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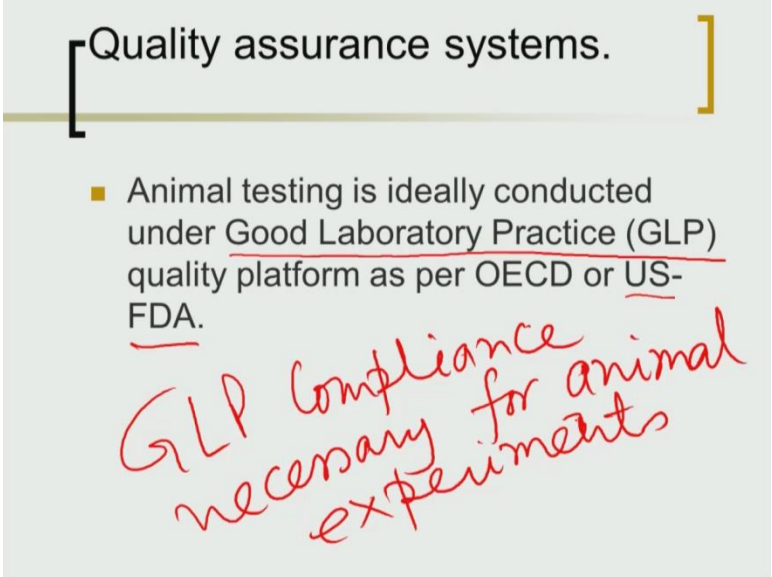

Okay, now ideally all the animal testing conducted under Good Laboratory Practice GLP quality platform as per US Federation of US FDA rules. The GLP compliance is necessary. So I need to highlight this point that GLP compliance necessary for animal experiments.

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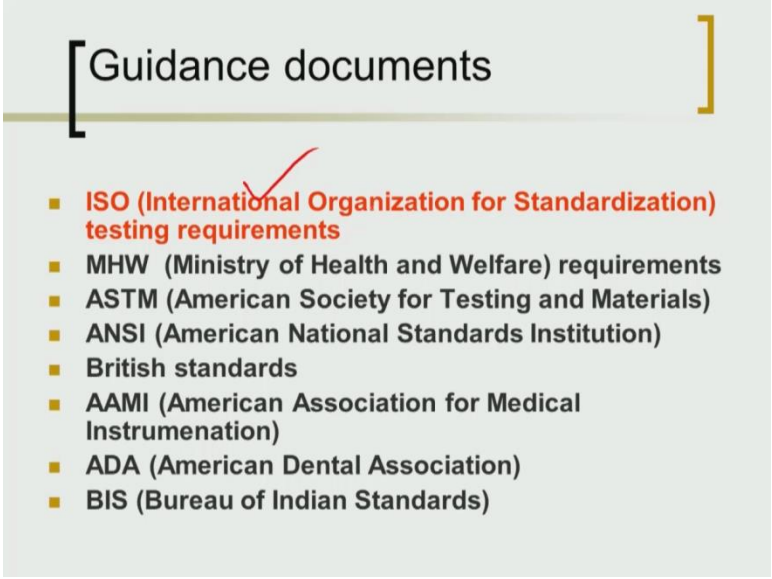

Okay. Now these guidance documents, there are several guidance documents and various countries they have their own. For example ASTM American Society for Testing on Materials, ANSI that is American National Standards Institution, you have British Standards, you have British Standards you have ADA, American Dental Association and we also have BIS that is

Bureau of Indian Standards. Again I have emphasised the first one that is ISO which stands for International Organisation for Standardization and ISO testing requirements is by far the most widely used in animal pre-clinical studies.

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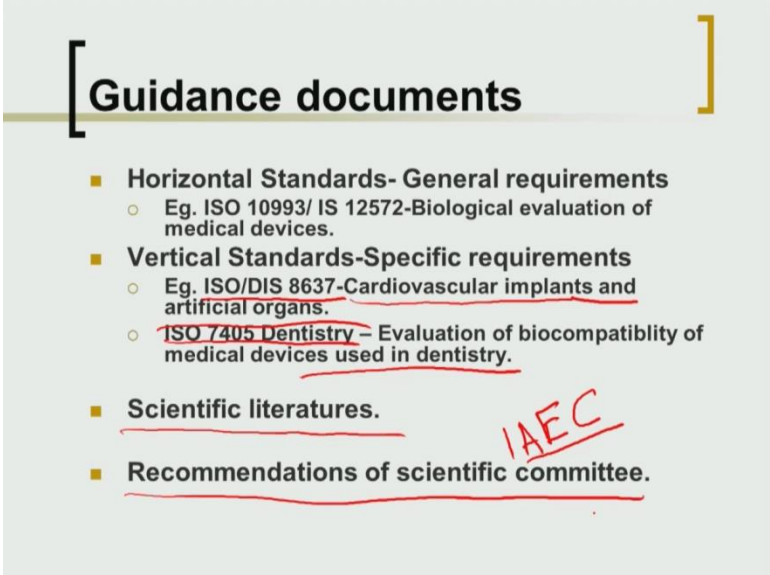

Now there are different, depending on what kind of biomedical application you are targeting. For example if you are interested for cardiovascular applications or cardiovascular implants and artificial organs, then you have to follow ISO/DIS 8637. If you are interested for Dentistry, Orthodontic or prosthodontics kind of applications then you can use the guidelines ISO 7405.

Apart from that published literature which are kind of available in PR reviewed journals, so those kind of literature also can be used to study, to design the animal experiments and recommendation of scientific committee like Institutional Animal Ethical Committee IAEC, they can also propose certain guide lines to modify or to add to the proposal that an investigator has submitted for (approve) So those recommendations also need to be taken care of before conducting these animal experiments.

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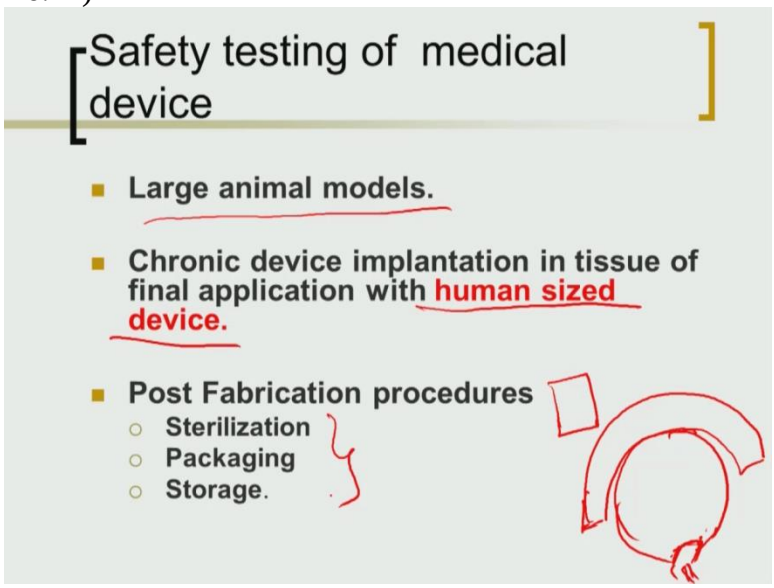

So one of the things people do to at the very beginning is to just to see to assess the biological safety of any medical, medical devices or large animals or large material devices and for that it is recommended to use large animal models and this is the chronic device implantation in the tissue of final application with human size devices. Now why I am kind of emphasising with human size device now normally in the laboratory scale always we use some scale down samples or samples of simple geometry or smaller in sizes.

Now human device sizes for example if you are interested on acetabular socket then human device size you have to use the same device size as well as fumoral ball head of same size. Ok? And therefore it is important to use same device size so that, that acceptance of that human size device can be better assessed in a relatively large animal model. Since you are using human size device one cannot use small animal models in this particular case. Now post fabrication procedures before, before this material can be put into human patients that involve sterilisation, packaging and storage Sterilisation can be done in different ways like UV sterilisation and so on. So I think I will stop now and then I will start with this next one in the next module.