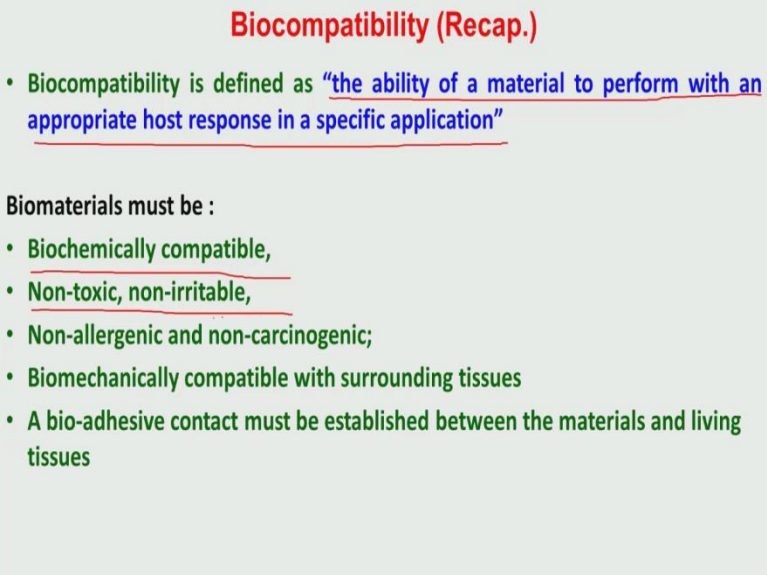
Biomaterials for Bone Tissue Engineering Applications
Professor Bikramjit Basu
Materials Research Centre
Indian Institute of Science Bangalore
Module 5
Lecture No 19

Ya, so we will start with specific discussion on in vivo biocompatibility assessment, with reference to the following points, selection of animal model, selection of a defect model and design of a study together with the qualitative and quantitative determination of biocompatibility.

(Refer Slide Time: 0:49)

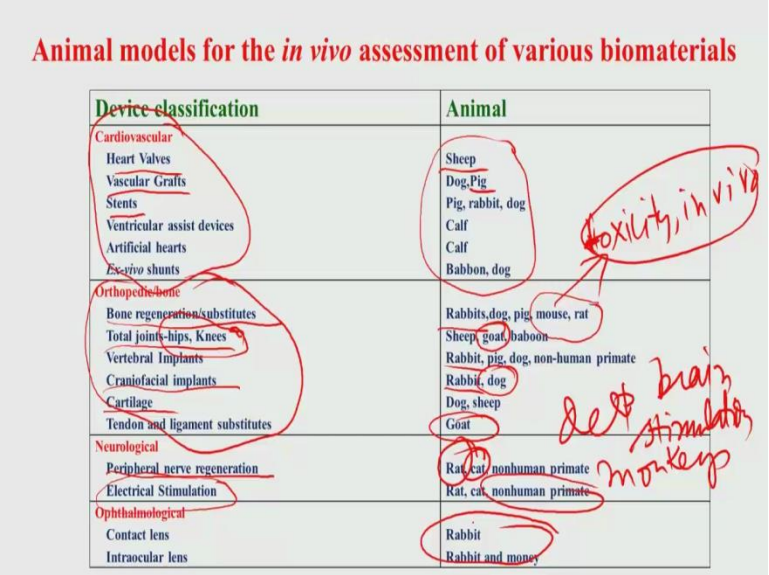


Now if you recall the biocompatibility definition, this is the ability of a, this is the ability of a material to perform with an appropriate host response, in a specific application. And this has been mentioned time and again earlier, that biocompatibility is a application specific property. And in general it has been widely understood, that biomaterials must be biochemically compatible. With the host tissue it should be non-toxic and non-irritable, non-allergenic and non-carcinogenic and biomechanically compatible with the surrounding tissue that is very important.

And here one of the things that I have mentioned earlier, that is the elastic modulus property is important, and because elastic modular if there is a large difference in the elastic modulus between the biomaterial and the surrounding tissue then that can cause aseptic loosening. And

overall there is a general consensus that bio adhesive contact must be established between the materials and the living tissue.

(Refer Slide Time: 1:49)



Okay. What are the different animal models that are what are the different animal models that are currently used for different implant, for different in vivo Study. So this table actually summarises some of the turn application specific animal model selection. In the case of cardio vascular applications like heart valve or vascular graphs or cardiovascular stents like made of nickel titanium or ventricular assist devises or artificial heart sense and so on, the largely people prefer or it is reported in scientific literature, that researchers use either pig model or sheep model.

So these pig model or sheep model these are the most widely used animal models for cardiovascular applications. In orthopaedic or bone replacement applications, for bone regeneration substitute, total hip joint or knee joint surgery, vertebral implants, craniofacial implants or cartilage applications, different models people have used but largely it is reported rabbit model in this you can see rabbit model are used extensively and reported in the literature

The next level of model after the rabbit, if the material is found to be bio compatible so one of the things that people use more often when they validate the biocompatibility testing of the lower animal in the higher animal and for that people can use goat model or dog model. So goat and

dog model is the next level of model that people use. For toxicity assessment of the biomaterials for example in the total hip and knee joints, your (())(03:50) particles are generated.

Now these particles when they are transported to different parts of the osseous structures, they can cause toxicity to the neighbouring tissue. Now for the toxicity of the small nanoparticle (())(04:05), then people use the smaller animals like rat model or mouse model. So this is essentially used for the toxicity inhibo. So, you can use you can use different experiment for toxicity, cytotoxicity property in vitro, similarly toxicity property also can be assed inhibo.

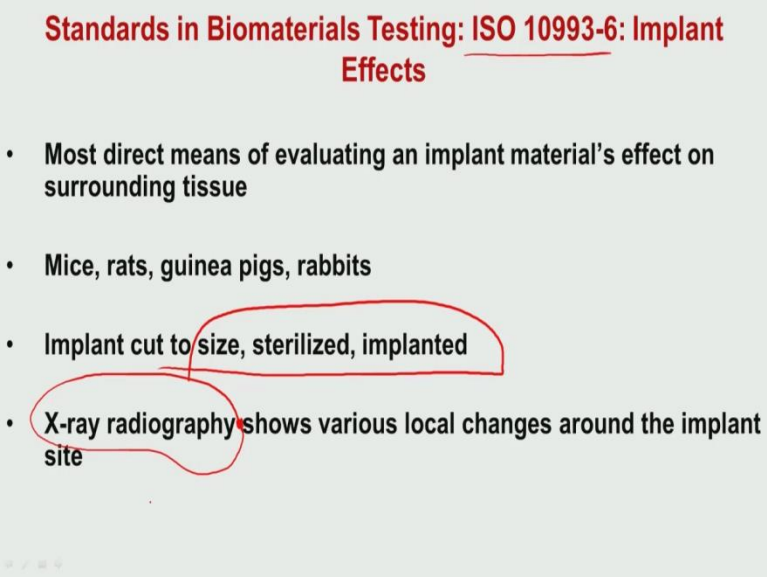
Third level of application that has been mentioned or summarised in this table is neurological. Now neurological there are two types of implantation experiments people have done, for example one can use the conduit nerve, nerve conduit for peripheral nerve regeneration application. So there even small animals like rat or cat model is useful. People use deep brain stimulation surgery.

Now in the deep brain stimulation experiments, in deep brain stimulation experiment people can use non-human primates like monkeys for example. So monkeys are more used in the disciple of neuroscience and so on and they are used for deep brain stimulation or other kind of neurological stimulation experiments and see the response of the animals, response of the primates to their stimulation.

The fourth one is the ophthalmological application like contact lens PMMA for example or intraocular lens. So there people use mostly rabbit model. So over all if you see the smaller animal models like mouse and rat, they are used more for toxicity inhibo study but for the cardio vascular applications people use, largely people prefer or researchers prefer to use large animal models like pig and sheep and for orthopaedic or bone regeneration mostly the rabbit and in higher level animal dog and goat model also is used for orthopaedic application.

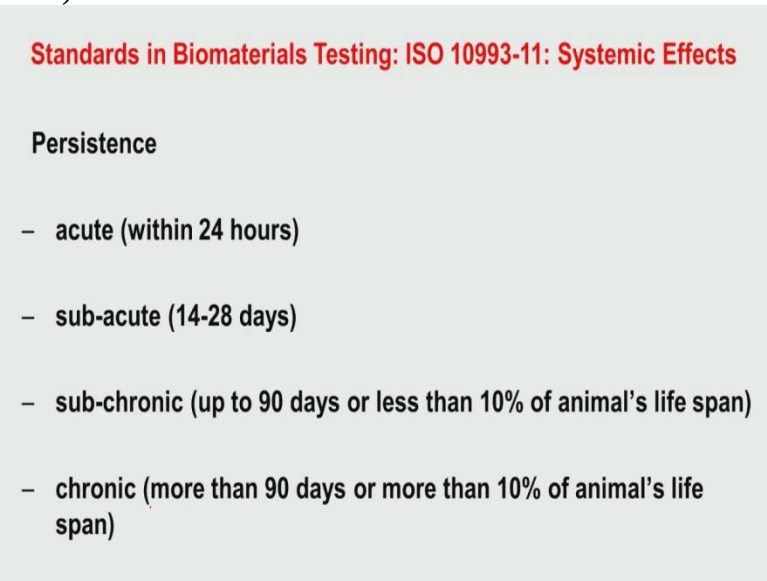
Neurological it is again rat model and non-human primates like (())(6:06) monkeys are used. For ophthalmological applications like you know eye related applications, contact lens and so on there you can use the rabbit model.

(Refer Slide Time: 6:14)



Now there are several standards like one of the standards for biocompatibility assessment is that ISO International standard organisation and ISO 10933 and part 6, that is that is essentially related to the implant effects and standards in biomaterial testing. So these essentially tells you most direct means of evaluating implant materials effects on surrounding tissue and different animals have to be used and they can be cut to size sterilised implants and implanted and extra radiography can be used as an immediate assessment technique just to see that how there is local changes around the implant side at the macroscopic scale.

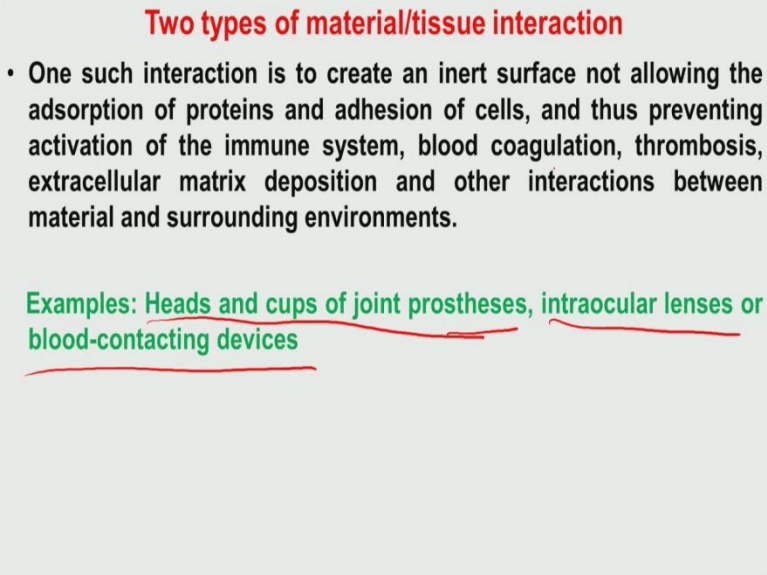
(Refer Slide Time: 7:01)



Now there are several, there are two important, two type of response, one is called acute response and one is called chronic response. So acute response is recorded this is a part of the systemic effect. Acute response typically is recorded within 24 hours. Chronic response chronic response in any animal model can be recorded in a time scale of more than 90 days or more than 10 percent of animal is life span.

Sub-acute is typically observed within 14 to 28 days that means 2-4 weeks. And sub chronic it is up to 90 days like three months' time or less than 10 percent of the animal is life span.

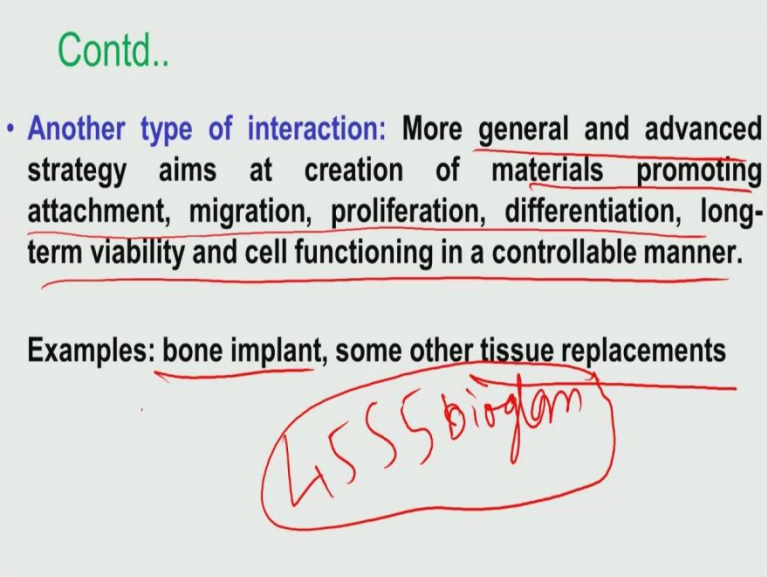
(Refer Slide Time: 7:48)



Ok now there are two types of material or tissue interaction which are important. One interaction is to create an inert surface not allowing the adsorption of protein and additional sub cells thus preventing activation of the immune system. And then examples of these types of material or tissue interaction is the heads and cups of joint prostheses, intraocular lenses or blood contacting devises.

So in those cases they essentially, the interaction with the host tissue essentially does not facilitate any biological tissue formation or very strong biological bone formation at the material interface.

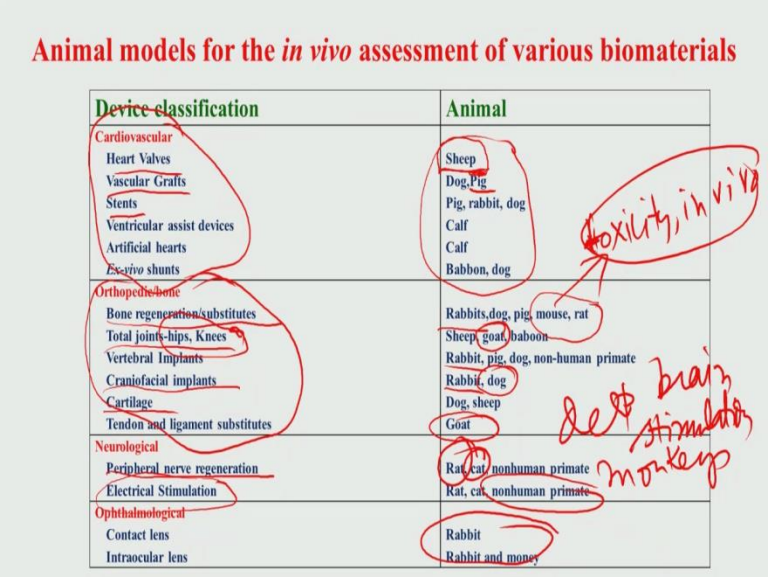
(Refer Slide Time: 8:34)



The another type of interaction it is more general or advanced strategy that aims at creation of materials which promotes the attachment, migration, proliferation and differentiation and long term viability and cell functioning in a controllable manner. And this is important for the bone regeneration purpose like bone implant and other tissue replacements. So one of the popular examples for that is 45s5 bioglass also.

So this is also one of the important material that which has been discovered by Professor Larry Henge few decades ago. So 45 percent silica, it has 45 percent silica that is 45S5, and there is certain ratio of phosphate P2O5 and (())(09:16) in this kind of glass materials.

(Refer Slide Time: 9:24)



So other things are important that in terms of the animal selection, is that why certain animals are preferred over lower order animals. Typical the one general principle that is observed in the pre-clinical testing is that if useful, or if a small animal model can serve your purpose, you should necessarily use the small animal model one should necessarily use small animal model over a large model, that is the number one point.

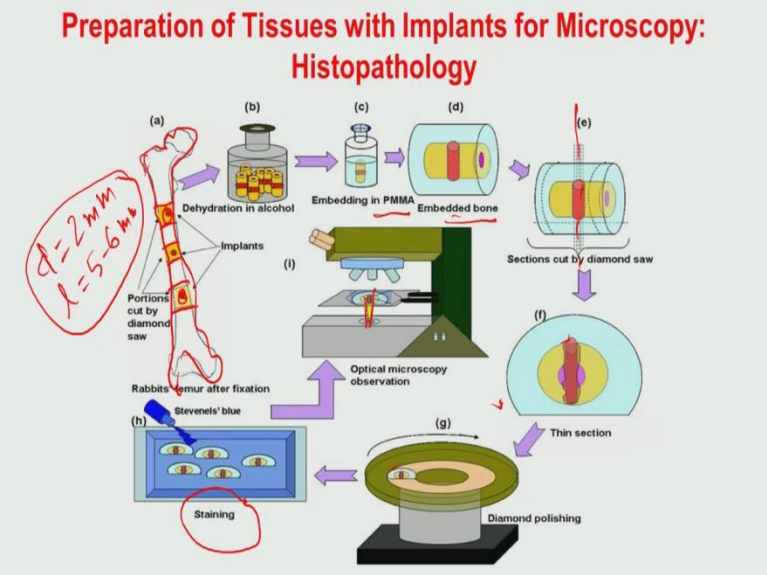
Number two point is that, if small animal gives you satisfactory biocompatibility response, then one has to validate that similar biocompatibility response or one has to validated the bio compatibility response of the same animal in the larger animal study. For example if some animal when implanted in the rat model or the mouse model gives good biocompatibility property the next level of experiment should involve higher animal like rabbit or goat or sheep model, before one can even think for going clinical trials in human patients.

Second, third consideration is that whenever you use certain animal model and you have to place certain material at certain anatomical location how easy to access that particular organs in that specific animals, that needs to be considered as well. For example if you want to use for cardio vascular applications the first primary consideration should be whether how easily you can accessed that animes heart so that you can put some cardiac patch or you can during the surgery you can place some artificial heart valves in that particular animal.

So if the animal is a very small animal then often it is very difficult to access that particular organ in that particular animal. So the accessibility to the organ in question or organ in terms of the particular specific application is also equally important. Fourth consideration and perhaps more scientific consideration is that how closely that animal bone structure, bone microstructure, bone macrostructure and bone properties or bone remodelling properties can match with that of that human system.

For example if you take some rabbit femur. The rabbit is femur their load bearing capability, their bone remodelling properties everything, whether how close it is goes to that of the human femurs. So these kinds of questions needs to be critically thought or needs to be critically answered while designing the animal experiments.

(Refer Slide Time: 12:18)



Ok, more discussion on the biological response of the implants. So this is your left hand side this is that implant materials which is placed in certain anatomical locations of an animal and this is your time T equal to 0. And this that X axis the time scale, and this time you can consider it is terms of days because most of the biological events that is taking place and the implant bone interface that are essentially kinetically quite slow process.

Now at the interface what you can see that is a neutrophil activity. Now just few in the last module itself I have discussed about the blood level compatibility of an synthetic animals blood

level compatible synthetic implants. So therefore again blood compatibility takes an important role simply because as soon as the material is implanted in any animal system or any living system, different blood elements, they come and interact with the material interface first.

So this is the neutrophils, you can see there and there is also monocytes that has been marked here. So these proteins are adsorbed on a biomaterial substrate and this has been, this is one of the pre curser for the cell attachment to the biomaterial substrate. So this protein absorption takes place within few hours of that implantation. Then after that you have fibroblast cells, because fibroblast cells is the connecting tissue cells, it forms connective tissue.

This fibroblast cell they come and adhere in the material substrate and that is the reason why fibroblast cells are used more widely and more extensively in screening biomaterials at the in vitro level. I repeat, fibroblast cell is one of the widely used cell line which researchers use while assessing that in vitro cytocompatibility property of any biomaterial because during wound healing process and during various implantation in that animal model fibroblast cells are known to interact with the materials as the first cell line which interacts with that synthetic materials.

So therefore it is important to see, how a material supports the growth and proliferation on the fibroblast cells. Ok then comes the matrix deposition. Now in the matrix deposition means, one fibroblast cell which you can see there is a very characteristic elongated kind of morphology or star shaped morphology, mono nuclear cells. And then once fibroblast cells then adhere then what happens? Then they also secrete some protein molecules and some biological micro molecules and that will help to synthesise exocellular matrix.

And this exocellular matrix you can see essentially this collagen fiber and elastane and this collagen fibers they are dispersed in the matrix and this collagen fibers provide kind of mechanical support to this or structural support to the fibroblast cells. There is also another thing has been shown in this slide, it is macrophage activity. That is also equally important. And towards the later time, or the implantation or the post implantation you also see multinucleated foreign body giant cells.

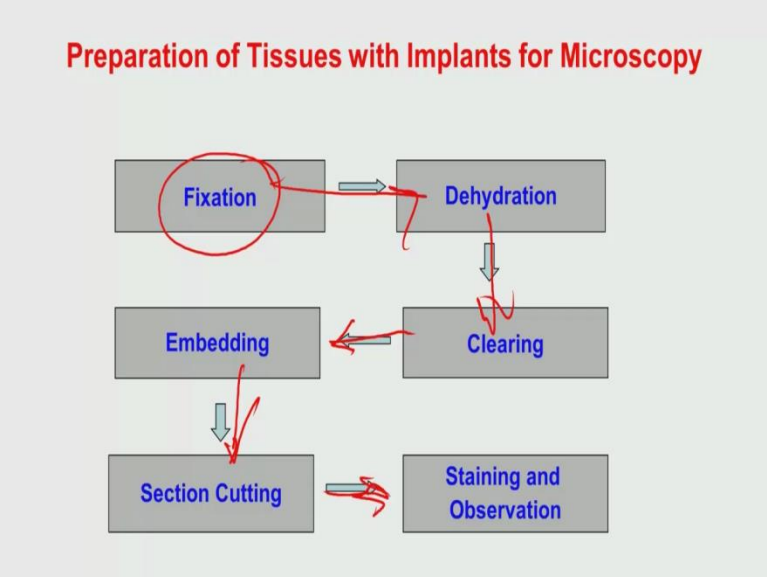
FBGC stands for foreign body giant cells. So this multinucleated foreign body giant cells, they also becomes activated. Now this entire process you can see right from the T equal to 0 to T

equal to 3 to 4 weeks' time period , up to 3-4 weeks, this entire process takes place and what is the net result? Net result is that there is something called fibrous encapsulation.

That means all these biological component, this fibroblast, monocytes, exocellular matrix neutrophils they kind of make a kind of shielding around the biomaterial interface at the biomaterial surface and as a result these biomaterial itself, which is marked as a star will not have any direct biological interaction with rest of the tissues or least rest of the components of the living system while it is implanted.

In other words, instead this biomaterial is no seeded from the rest of the osseous system with the formation of this fibrous capsule on the material surface.

(Refer Slide Time: 17:10)



Now after this showing this, this biomaterial, this various biological events at the material substrate, the next thing that I have shown you that you know that within two weeks of time frame after 12 to 14 days that what are the different stages that is taking place? One is the inflammation and second one is the repair. During repair how the different cells or proteins they increase in their numbers.

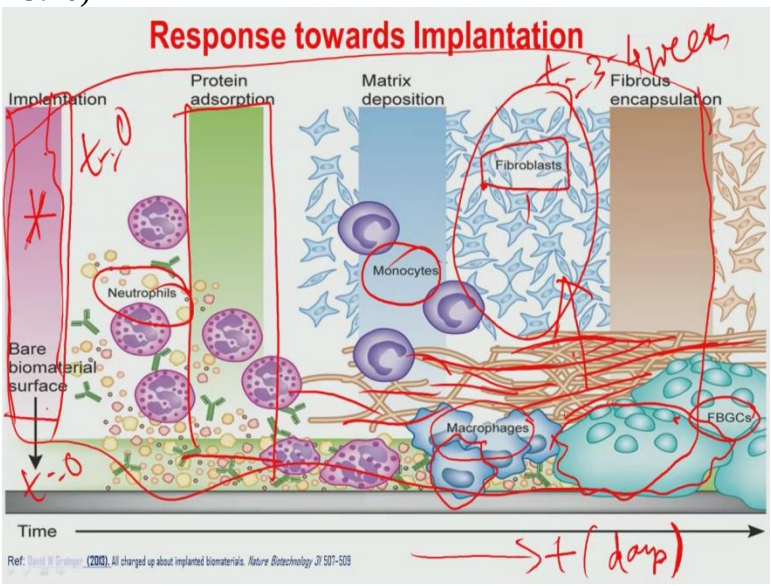
So if you start with, the neutrophil activity start to increase, this is in the arbitrary unit and then it goes saturated and then decreases. Then if you see monocytes, although they increase in number their numbers is much less than that of the neutrophils during the inflammation processes, and I

have mentioned in the last to last module also, that inflammation is one of the biological events which one cannot avoid irrespective of what kind of material that you put irrespective of whether you are putting it simple by well aseptic conditions during what kind of surgery that inflammation is bound to happen whenever any synthetic non-living material is being placed in an otherwise living system.

Third one is the fibroblast activity or a fibroblastic activity goes to a peak almost like near to one week time frame. Now collagen synthesis also starts off once this sufficient number of fibroblast and you can see there is certain kind of peak value of fibroblast and there the collagen synthesis also picked up and that increases further during the post implantation period. Blood capillaries formation also starts in the neutrophil activities other things, the blood capillaries also keeps on increasing picking up and that increases up to one week post implantation.

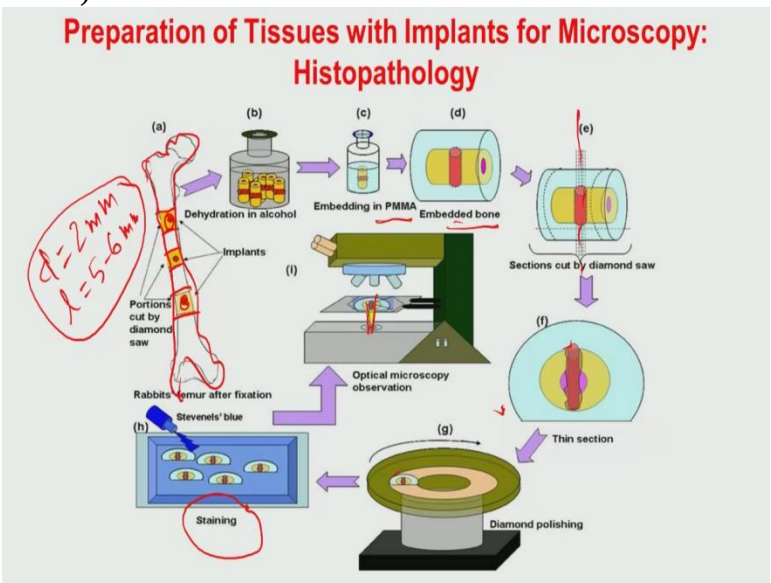
So once this inflammation period is over, then repair stage fibroblast activities goes slowly down with collagen activities (())(19:16) picked up and that helps in the tissue repair. So variation depends, this variation this temporal variation or this different acute increment response and repair response depends on the extend of the injury created during the implantation and the size, shape, topography chemical and physical properties of the biomaterials.

(Refer Slide Time: 19:40)



So that means that what is the size of the biomaterials like whether it is a segmental kind of defect or whether it is very small defect, what kind of surgery has been done during the implantation, what is the surface topography whether there is a smooth surface or whether it is a very rough surface, so all those properties will determine what is the temporal what is the temporal variation which I have explained to you in last 5 minutes or so.

(Refer Slide Time: 20:10)



Ok. So having said that these are all these cellular activities around the biomedical implants or what is the tissue response on these biomedical implants that needs to be carefully assessed using

certain microscopes which can enable you to identify different cell types in the tissues in the neighbourhood of the synthetic materials. Now this is possible only with the histological analysis. So histology means that you are trying to assess the structure of that tissues and cells that contained in it.

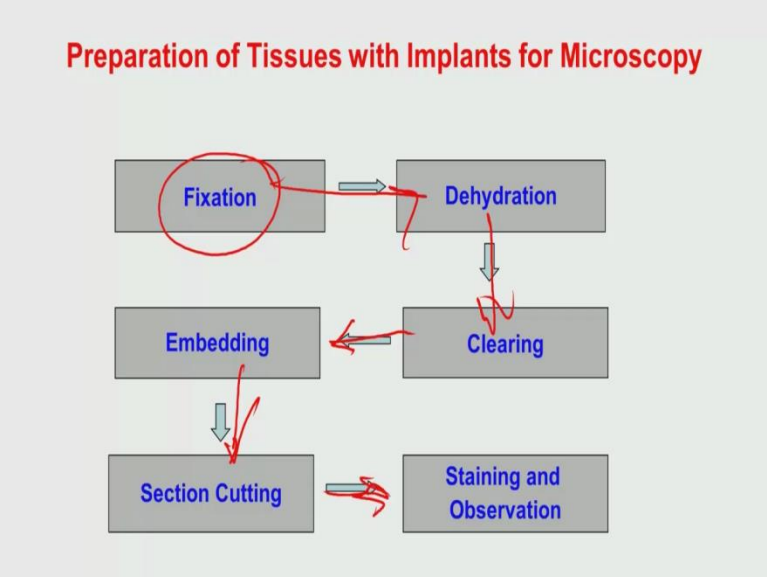
Now this is a typical example of a long bone structure you can see. So in the long bone structure I have put some kind of holes here. So this is the defects that you are essentially creating. This is called fumoral defects. So you take rabbits femur then you make these three holes. Now typically the diameter of this hole is 2 millimetre. According to the ISO standards the length of the implant that you can put here is somewhere around 5-6 millimetre in length.

This is the typical International Standard organisation specified guidelines for the inhibo biocompatibility in the rabbit model.Now once you make a hole insert it and then you can do this experiments, you can close the wound and then you can allow the rabbit to live for certain period of time like one week, 4 week and 12 weeks and so on, then after that you can do a series of dehydration in the explants what you call.

This is implants, then it is taken off, then you do all the kind of chemical treatments embedded in the poly methyl methacrylate resin, then embedded in bone, then you make thin sections of the bone and thin sections you can polish it in a diamond paste on a polishing wheels then after that you can stain the histology section. Now the staining can be done using different histological stains depending on what part of the tissue structure you want to reveal or in other words which cells in the tissue you want to reveal.

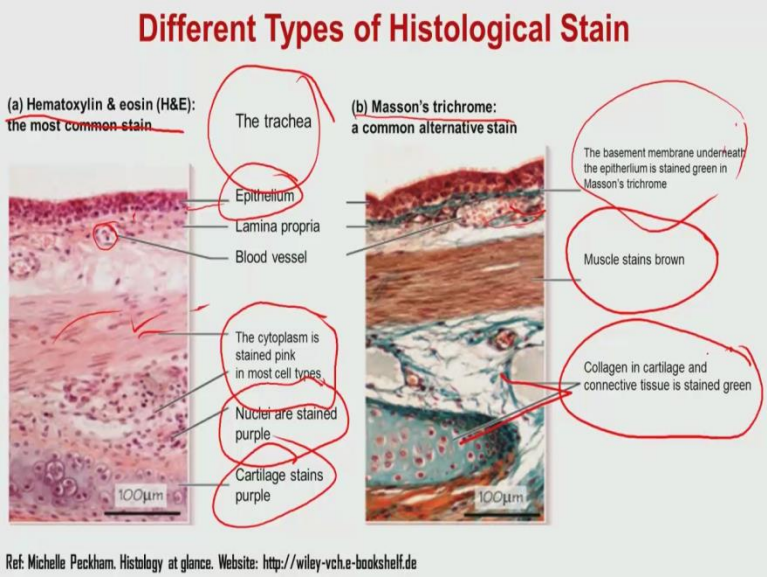
One of the staining agents that is quite commonly used is the (())(22:30) staining . And then after that you can do optical microscopy observations.

(Refer Slide Time: 22:39)



So essentially first you take the explants, you fix it, you dehydrate you clean it. Then you embed it in poly methyl methacrylate, then you cut the section and then after that you do the staining and observations.

(Refer Slide Time: 22:52)

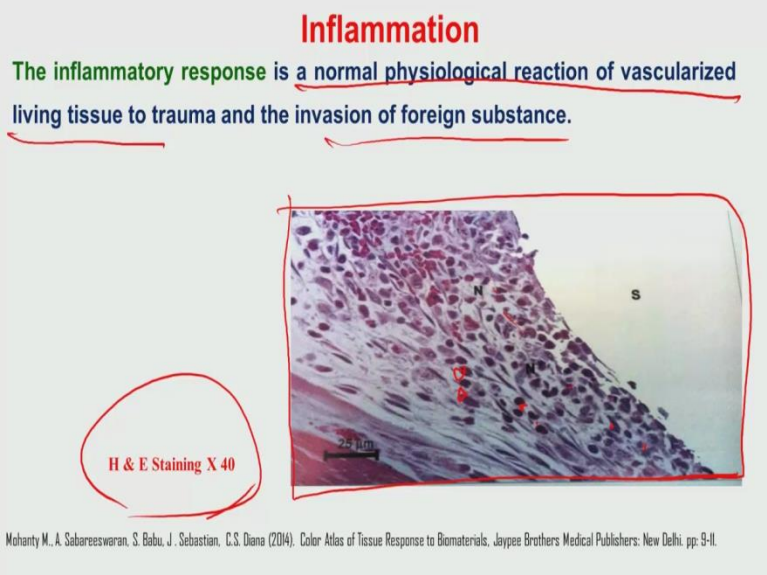


Now this is the typical histological stains and these histological stains can be used depending on what kind of tissues that you want to see. One of the commonly used histological stains is haematoxylin and eosin and second one is the Masson is trichrome stain. This is an alternative stain. Now what are the things that you want to see more clearly? This is the blood vessels here

and this is also the epithelial you can clearly see, the cytoplasm is stained pink in most of the cell types.

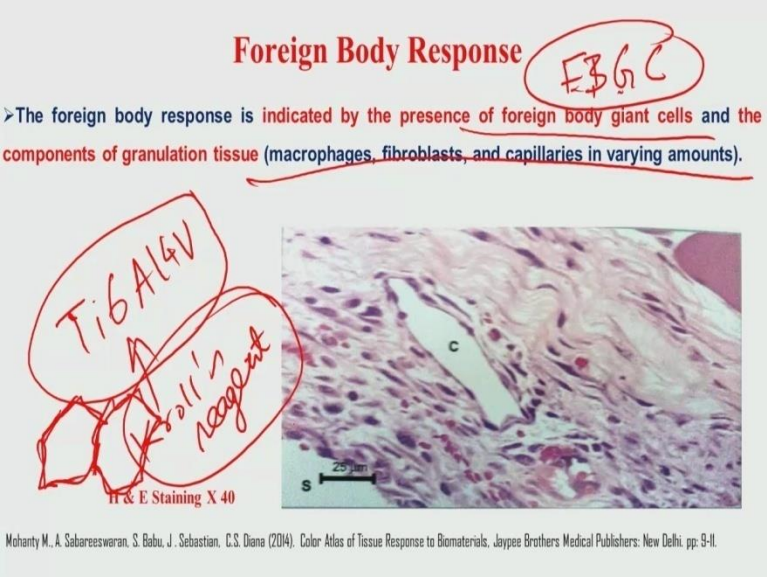
This is the cytoplasm stain and nucleus stained purple and this cartilage is also stained purple. In the Masson is trichrome staining of the same trachea, which you have seen here, the same thing we are trying to see. Here Masson is stain is brown and the also epithelium is stained green in this Masson is trichrome stain. Other things you can see is the collagen also, and collagen in cartilage and connecting tissue is stained green. So these two stains are more widely used.

(Refer Slide Time: 23:55)



This is again one of the haematoxylin and eosin stained image in 40 times magnification. This is taken from Colour atlas of tissue response to biomaterials which is written by Meera Mohanty and ())(24:08) from ())(24:102) medical science and technology. So we have taken this section from their book then you can see that how this inflammatory response that is which is a normal physiological reaction of a vascularised living tissue to trauma and essentially it facilitates the invasion of the foreign substance. And you can see that what are the different cells that which are participating in this inflammatory response.

(Refer Slide Time: 24:40)



The additives is equally important to understand this the foreign body response. So this foreign body response is indicated by the presence of what I have told you before, foreign body giant cells. This foreign body giant cells is essentially multinucleated cells and components of the granulation tissue like macrophages like fibroblast or capillaries in varying amount.

So macrophages fibroblast means that is the collecting tissue cells which are blood capillaries in varying amounts also should be present and this entire things again you can study from the tissue section and tissue section needs to be stained by haematoxylin and eosin which is a staining agent. Now if you are from material science background the staining can be better realised as equivalent to agent what people use in the microscopy sample preparation for material science.

So agent is a chemical agent for example, if you use titanium material or Titanium 6 aluminium 4 vanadium material this material once you need to try to find out what is the grain size of the material you use certain reagents called kroll is reagent. So let me not get into more details of what is the composition with Kroll is reagent and all but for the time being you realise that kroll is reagent is a chemical solution.

This chemical solution etches the material and differential etching at the grain boundary region and around the grain will make you distinguish the grain boundaries from the rest of the material surfaces. So essentially you can see very nicely hexagonal grains or whatever size and shape of

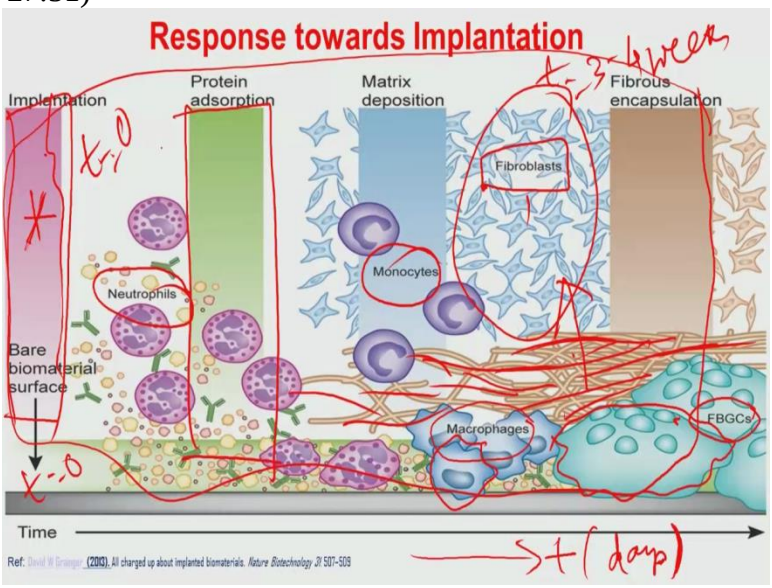
the grains that you can find in the material and this grain boundary region is etched differently than rest of the grain body whatever reagents you are using.

Now taking this concept back to that kind of histological analysis you can see for that you are using haematoxylin and eosin or Masson is trichrome. This is also certain reagents and that kind of helps you to identify different tissue components here.

Now tissue component are certainly very different type of structures all together from a general material whether it is titanium copper or stainless steel and so on. So compared to that it is a material you do not see any garn structure like this stop however you do see different kind of cells which has different morphology in the tissue and this particular reagents help to distinguish the different cells which are contained or different cellular features which are contained in the tissue structure.

Ok so I hope I have been able to kind of make you understand that the use of different staining agents in the histology sections and different cellular morphology they would appear n different features and that will help you to understand that what kind of cellular activity is seen in the particular tissue response.

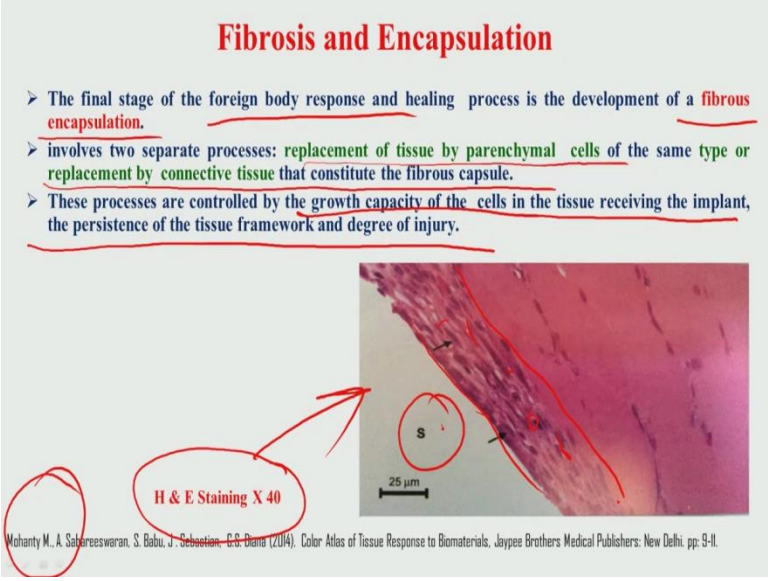
(Refer Slide Time: 27:51)



The final stage in the foreign body response and the healing process is the development of fibrous encapsulation. And this fibrous encapsulation process I think I have spend sufficient time

in this particular slide to explain you what is meant by fibrous encapsulation and what are the events that finally lead to the this final particular biological event leading to the fibrous encapsulation.

(Refer Slide Time: 28:27)



Now this fibrous encapsulation also involves you know that activity towards the end stage of the fibrous encapsulation is the forint body giant cells and that has been mentioned here that fibrosis and fibrous encapsulation this is two separate processes one is the replacement of tissue by parenchymal cells of the same type and second one is the replacement by connecting tissue that constitutes the fibrous capsule itself.

So these independent processes they are controlled by the growth capacity of the cells and tissues receiving the implant and the persistence of the tissue framework and also to some extend to the degree of injury. Now this has been mentioned before that depending on what is the kind of surgery that you are using, depending on how severely that wound has taken place, even if you are putting the material into this fibrosis and fibrous encapsulation process taking place in different extent.

So this is again last example, this is again haematoxylin and eosin stained image which is again taken from Meera Mohanty is book on Colour atlas of tissue response to biomaterials and you can see this tissue response, you can see that here how different cells are aligned in this, this is

the sections and on which you have used the stains and this fibrosis encapsulation can be confirmed from histological analysis itself.

So I think I would stop now and so next I will continue very briefly on this tissue response again and after that we will go to the different topic as part of this course.