Oral Biology
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Lecture - 11
Mineralization dynamics - Part 2

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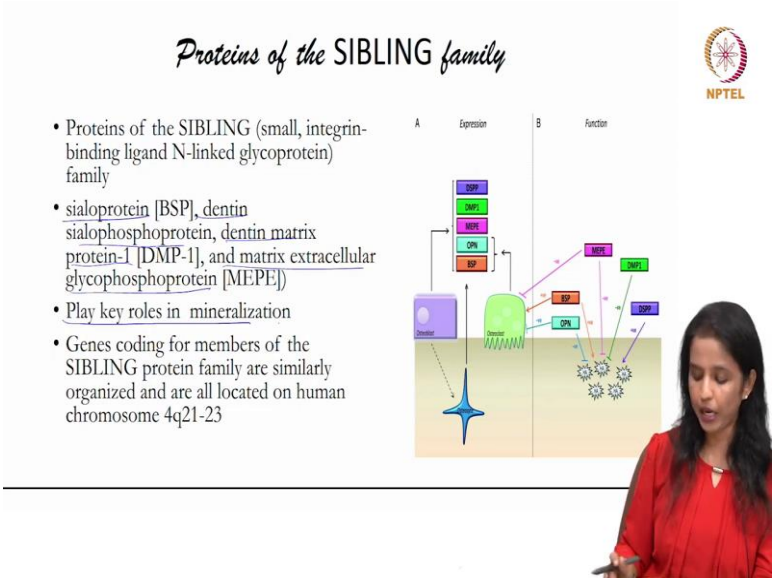

Next, we move on to the proteins of the SIBLING family. So, these again are the promoter part of mineralization agent, promoting agents. So, the examples of the promoting agents are sialoprotein, dentin sialophosphoprotein, dentin matrix protein and matrix extracellular glyco phospho protein. So, these actually are very important group which are actually grouped as the SIBLING family which is the expand when short version acronym of small integrin binding ligand and like linked glycoprotein.

So, SIBLING stands for small integrin binding ligand N-linked glycoprotein and these are actually very important and play a key role in the mineralization process.

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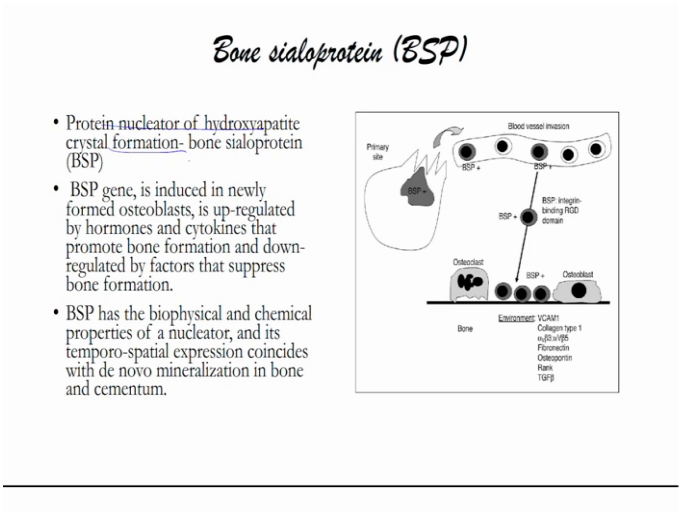

So, among the SIBLING family, bone sialoprotein is very important and this bone sialoprotein acts as a nucleator of hydroxyapatite crystal formation. We saw that in heterogeneous nucleation. You need a presence of a nucleator for the mineralization to happen. So, this bone sialoprotein acts as a nucleator to initiate that beautiful process of mineralization and this the bone sialoprotein is induced in newly formed osteoblasts and up regulated by hormones and cytokines.

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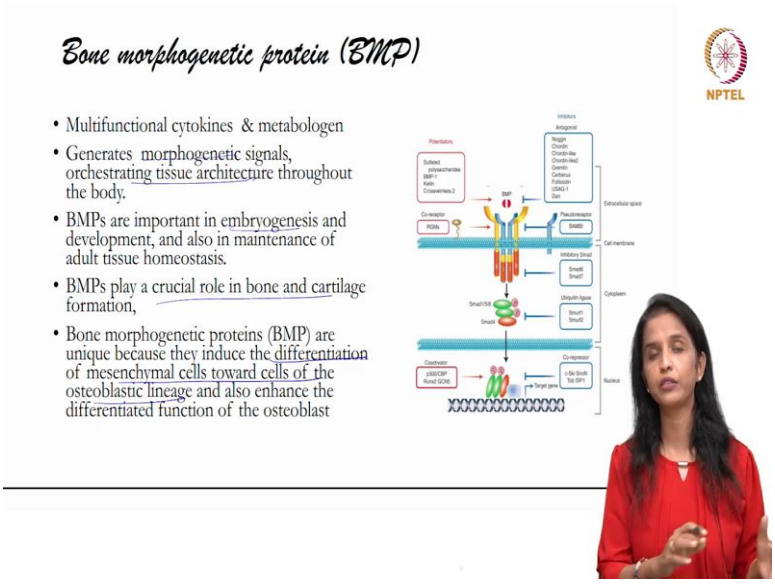

And further we have bone morphogenetic protein and this is actually a multifunctional cytokine and also called as a metabologen because it actually generates many morphogenetic signals as the name suggests and it also orchestrates tissue architecture. Hence, the morphology the executing the morphological aspects, important in embryogenesis and development and plays a crucial role in bone and cartilage formation.

So, very important and main thing is they actually trigger the differentiation of mesenchymal cells toward cells of the osteoblastic lineage. For example if there is an undifferentiated progenitor cell, the BMP would trigger them to become a osteo blast.

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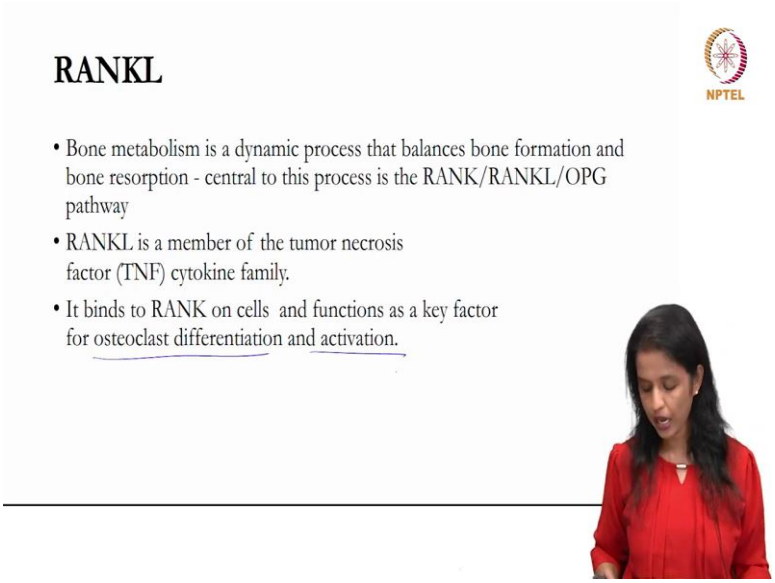

And then we go on to see the RANKL. RANKL actually is again a very important member of the tumor necrosis family and is a key factor in osteoclast differentiation and activation.

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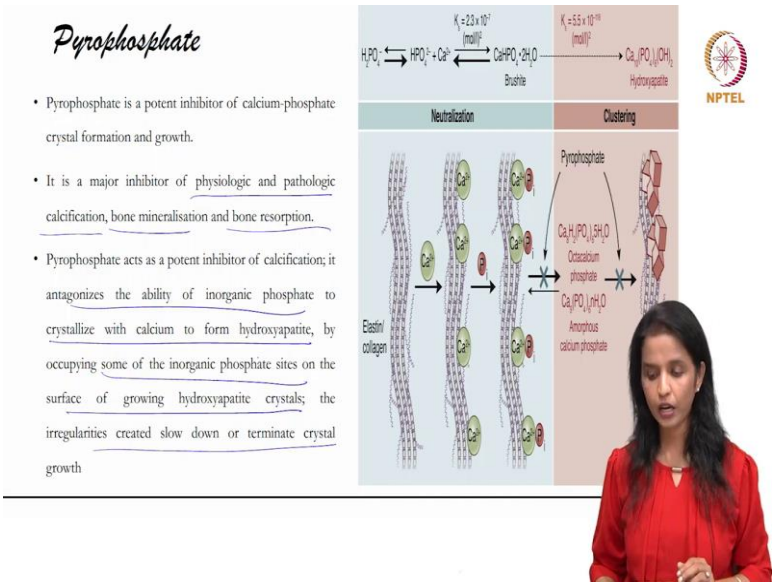

And further we move on to pyrophosphate which is an inhibitor of mineralization, a very important inhibitor. So, it is actually called as a potent inhibitor of calcium phosphate crystal formation and growth. So, the major inhibitor of physiological and pathological calcification, bone mineralization and bone resorption.

Having heard that statement, why should mineralization be inhibited when we know that it is very important to form hard tissues and makes us more what to say fit and more harder, more stronger, more healthier, but it is not like that we do not want mineralization in areas where soft tissues need to be. So, those areas need not have to be mineralized, need not have to be hardened.

that particular mechanism is actually handled by the presence of inhibitors and pyrophosphate is one such very important inhibitor which is vital for inhibiting the unnecessary, uneventful mineralization from happening. So, how does this happen? it actually antagonizes the ability of inorganic phosphate to crystallize with calcium to form hydroxyapatite.

this I mean by this is actually happening by the inorganic phosphate sites on the surface of growing hydroxyapatite crystals. The irregularities created slow down or terminated by the crystal growth. So, what exactly happens is, there is an antagonistic activity by binding to the inorganic phosphate ability to crystallize with the calcium to form

hydroxyapatite. So, it acts on the phosphate not allowing that to become hydroxyapatite along with your calcium.

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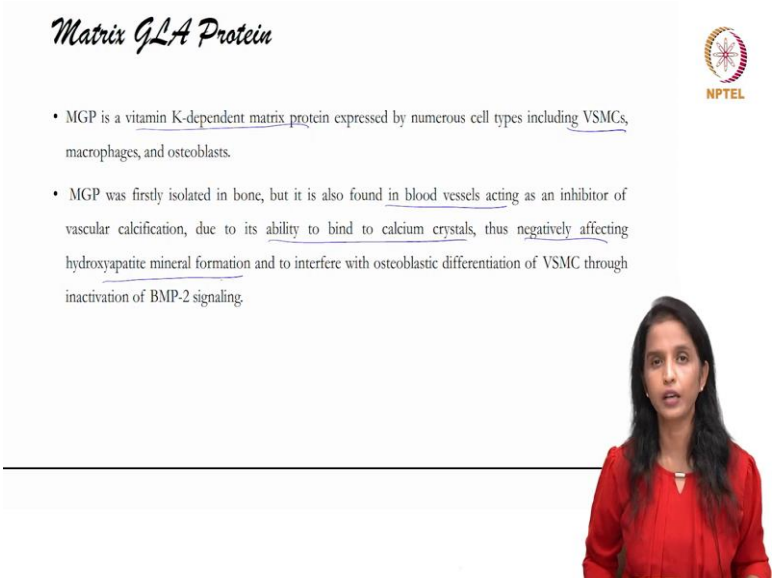

And then the next inhibitor, next to pyrophosphate ion matrix GLA proteins. So, matrix GLA proteins is vitamin K dependent matrix proteins and expressed by numerous cell types including your stromal, stem cells, macrophages and your osteoblast. So, this matrix GLA proteins are actually found in blood vessels and they are very important because they have the ability to bind to calcium crystals and negatively affects hydroxyapatite mineral formation and interferes with osteoblastic differentiation. So, this is a very important inhibitor, next to your pyrophosphate.

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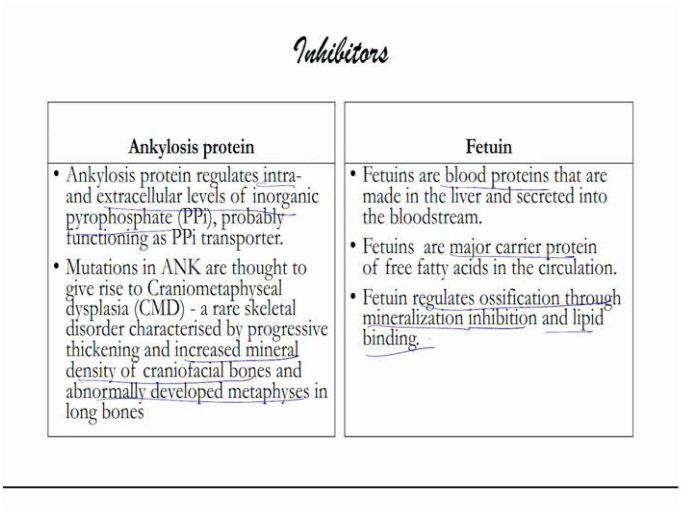

The other inhibitors which have to be known are your ankylosis protein. Ankylosis protein are the ones which actually help the pyrophosphate to do its action. So, how does it help? It actually regulates the intra and extracellular levels of pyrophosphate by acting as a pyrophosphate transporter. So, that is what the ankylosis protein does and by bringing it inside the cell and outside the cell what happens is, it executes or controls the inhibitory activity of pyrophosphate.

In another word, it actually helps in or aids in the pyrophosphate to do its activity and this was proved by checking mutated mice which actually if there was a mutation of ankylosis protein then it was found that those animals were actually having establishing a rare skeletal disorder called as cranio-metaphyseal dysplasia.

So, where you will have increased mineral density of craniofacial bones and abnormally developed the major part of the metaphyses of bones because the inhibitors were not there, the bone mass was very much higher than what is required.

Then we have another inhibitor here that is fetuin. So, this fetuin is actually a very important blood protein which are almost called as carrier proteins and the most important carrier protein which we all know is your albumin and that is actually important in regulating ossification through mineralization, inhibition and lipid binding. So, it actually acts as an inhibitor by inhibiting the mineralization through lipid binding.

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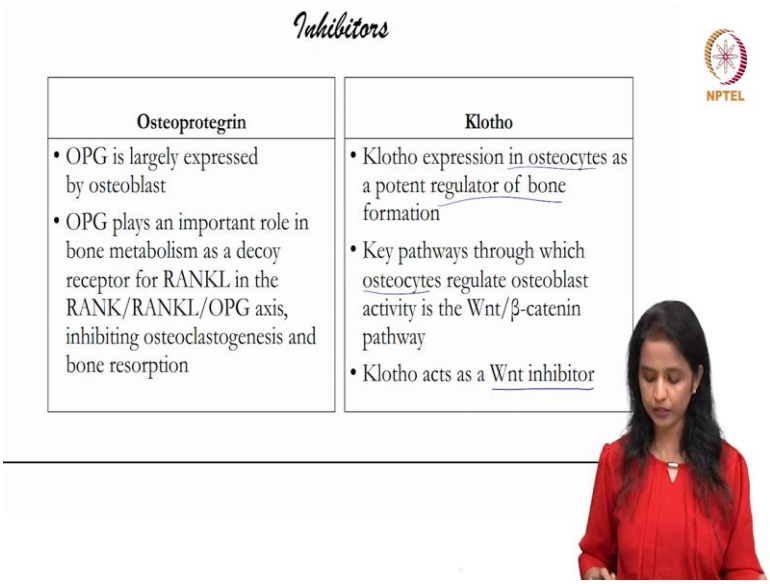

And further in addition to pyrophosphates we saw matrix GLA proteins, we saw ankylosis protein and then your fetuins, additionally we have osteoprotegrin and klotho. So, osteoprotegrin is actually secreted by the osteoblast, but again it also has an inhibitory activity when the amount of bone secretion is adequately formed and further there is a inhibitory activity which also is controlled by osteoprotegrin.

So, there is a promoter and her inhibitory role and in klotho, the klotho factor actually expresses in osteocytes and is a potent regular in bone formation. So, how does it regulate bone formation? So, the WNT pathway is actually the key pathway in osteocyte. We already know that osteocytes are resting bone cells and this actually plays a very important role in regulating the osteocytic activity and this acts as a WNT inhibitor and then we move on to theories of mineralization.

So, these theories of mineralization will actually help us to understand how the process of mineralization is happening.

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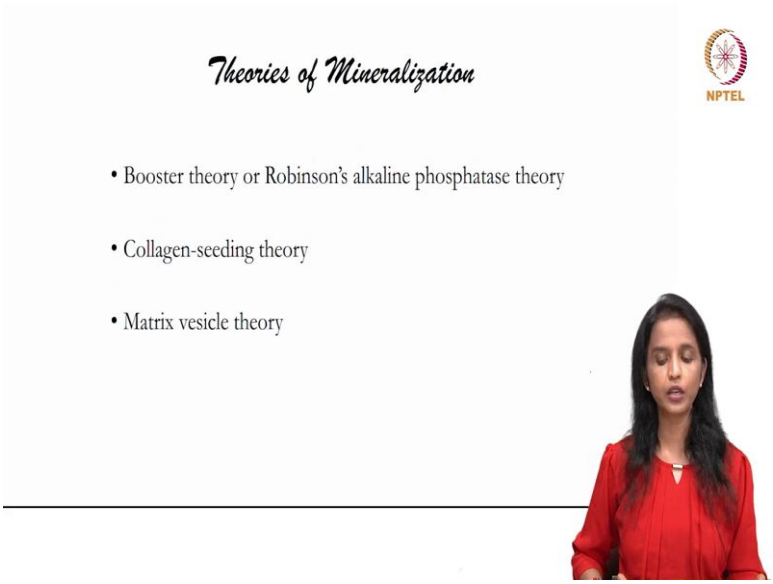

there as the as science kept evolving, the process of mineralization where understood by various theories which kept in involve evolving over the time and we have three theories and the most accepted theory here is your Matrix Vesicle Theory but we will just have a look at how the theories evolve and what are they to say to us to add on to learning of mineralization.

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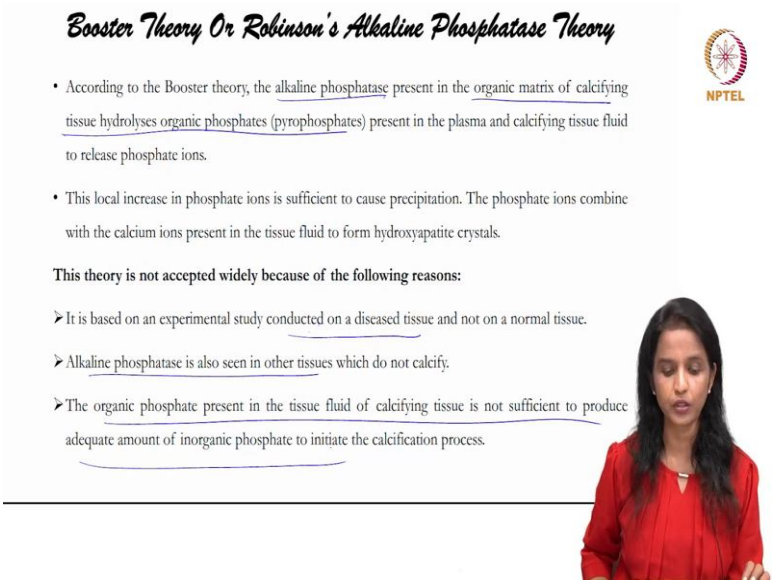

So, Booster's Theory or Robinson's Alkaline Phosphatase Theory as the name indicates that the alkaline phosphatase is the most important enzyme which is regulating the

mineralization event. So, alkaline phosphatase present in the organic matrix of calcium tissue actually hydrolyzes the pyrophosphates.

So, the mechanism of action which the alkaline phosphatase does is by inhibiting the pyrophosphates. By inhibiting the pyrophosphates what happens is, the inhibitor mechanism is stopped whereas, the promoter mechanisms take over and that is how it works and then the mineralization event happens.

So, this theory puts in that alkaline phosphatase has a more stronger or a powerful role to play in mineralization mechanism, but this was actually not accepted because this was actually, this was based on conducted on a study which was done on a diseased tissue and we all know that alkaline phosphatase is present in various other tissues as well.

So, when it is present through when in other tissues as well why is that particular area not getting mineralized and why is this getting mineralized? So, there were no adequate explanations for the same and organic phosphate present in the tissue fluid is not, I mean sufficient enough to produce adequate amount of inorganic phosphate to initiate the calcium phosphate, I mean the organization mineralization process.

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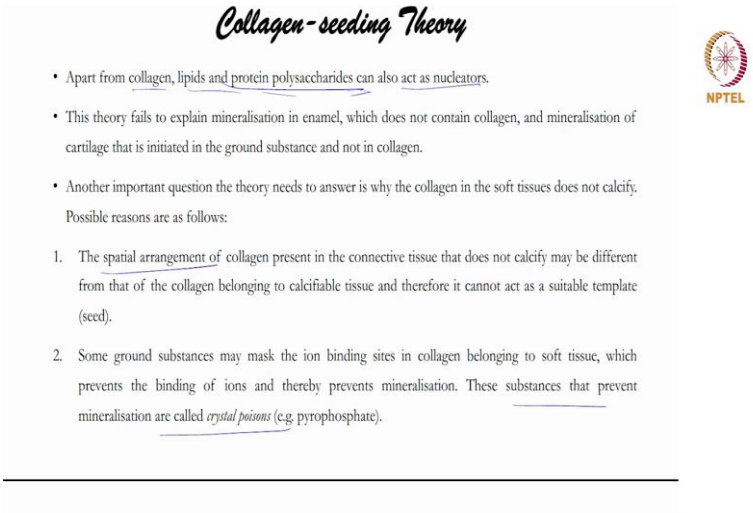

So, then we move on to Collagen Seeding Theory. So, here we know that collagen is the most important part of the organic component of your hard tissue. As already mentioned, type one collagen is the most important part of your mineralized tissues.

The collagen act as nucleators, but what happens is, they fail to explain the mineralization process in enamel because in enamel there is no collagen involved and there is no collagen at all. So, all other hard tissues that is your bone, the I mean dentin and the cementum, all have type one collagen, whereas, enamel does not have, but so this collagen seeding theory could not fit in the hard tissue, enamel and the other important theory is that we know that collagen is part of all soft tissue as well.

So, why is that not getting calcified? So, the explanation of this theory could be the collagen template, we know that it is a very important in the organic component is very important determinant, but the collagen organization which is present in the bone is different from what is present in the soft tissue.

So, because the spatial arrangement are different and additionally the substances which are preventing the mineralization that is inhibitors are not there in your soft tissues.

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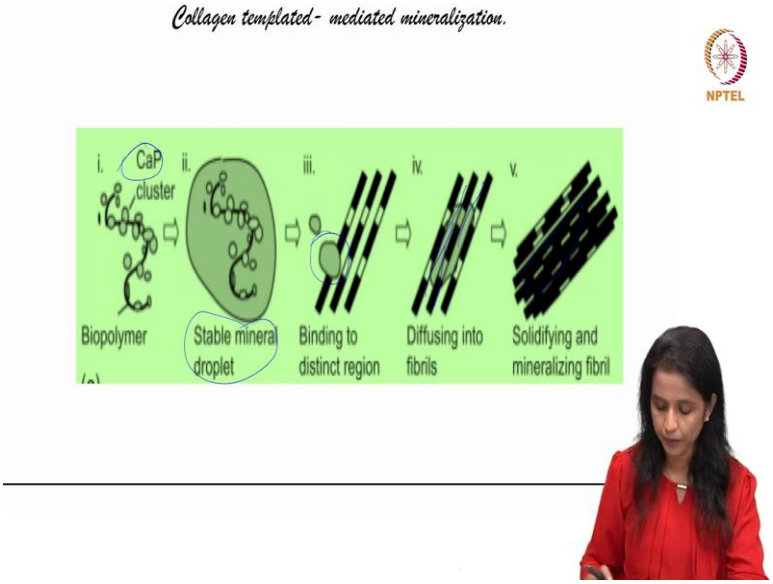

So, that is why you do not have the collagen which is becoming calcified.

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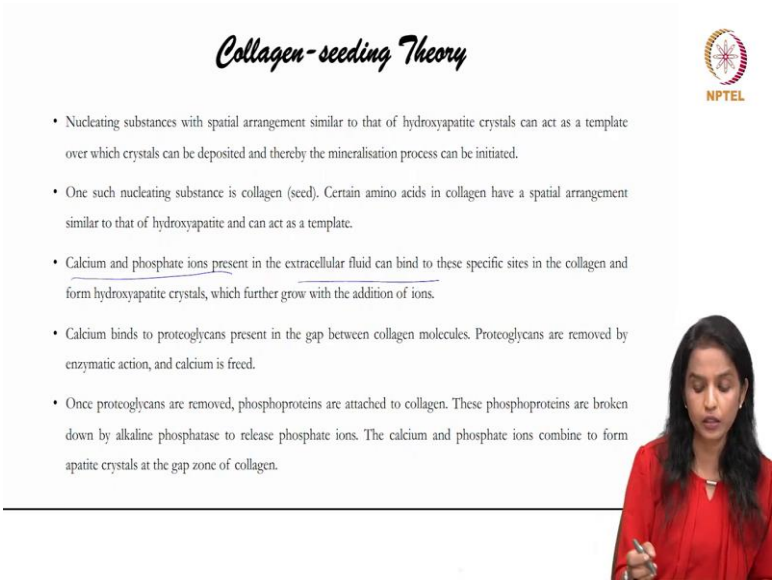

So, the nucleating substances similar to that of hydroxyapatite crystals also act as templates for mineralization process. So, that particular seeding agent, your collagen, presents with a spatial arrangement and that is very similar to what the hydroxyapatite crystal structure requires.

So, what exactly happens is, as the template is ready the collagen template is ready, the calcium and phosphate ions present in the extracellular fluid bind to these specific sites and then they act as nucleators and then further trigger the attraction of other ions in the neighboring extracellular fluid and then the nucleation even takes place and the nucleation begins and then it grows the crystal size increases in size.

And this is actually the schematic diagram for collagen templated mediated mineralization. We can see that you can the calcium phosphate clusters are there. The stable mineral droplet forms and then this goes and attaches to the binding region of the collagen fibers and further they diffuse and enter into the inter fibrillar region of the collagen template and all these together merges and then they solidify mineralizing the entire fibril.

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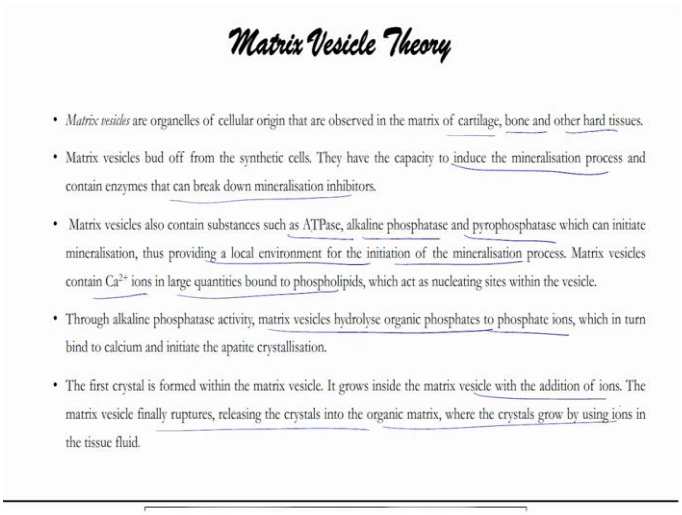

Then we move on to the most accepted theory that is your Matrix Vesicle Theory. So, matrix vesicles are as the name suggests they are small vesicles which are present in the matrix of cartilage, bone and other hard tissues. So, where do they come from?They are actually, what you say a budded or it just comes as budding out from the synthetic cells from the osteoblasts and so on.

And they actually act as inducing by my inducing the mineralization process and they contain enzymes which can actually break down the mineralization inhibitors which means that they will be having lactic enzymes good enough to break down the inhibitors. So, these matrix vesicles what do they contain? They contain ATPase.

They contain alkaline phosphatase which is the enzyme present in the osteoblast and pyrophosphatase. Please do recollect that we were telling about inhibitor pyrophosphate. So, there is an enzyme in the matrix vesicle which is called pyrophosphatase which is good enough to break down the pyrophosphate and thus giving out a inhibitory effect and this produces a local environment for initiation of mineralization process.

So, matrix vesicles contains calcium ions in large quantities bound to phospholipids which acts as nucleating sites within the vesicle. So, both in spite of this alkaline phosphatase activity, matrix vesicles hydrolyzes organic phosphates to phosphate ions which again further help in strengthening the hydroxyapatite crystals and that thus initiating the apatite crystallization.

So, the first crystal is actually getting initiated in the matrix vesicle and it grows inside the cycle with addition of ions and then it finally, ruptures releasing the crystals into the organic matrix which again keeps growing.

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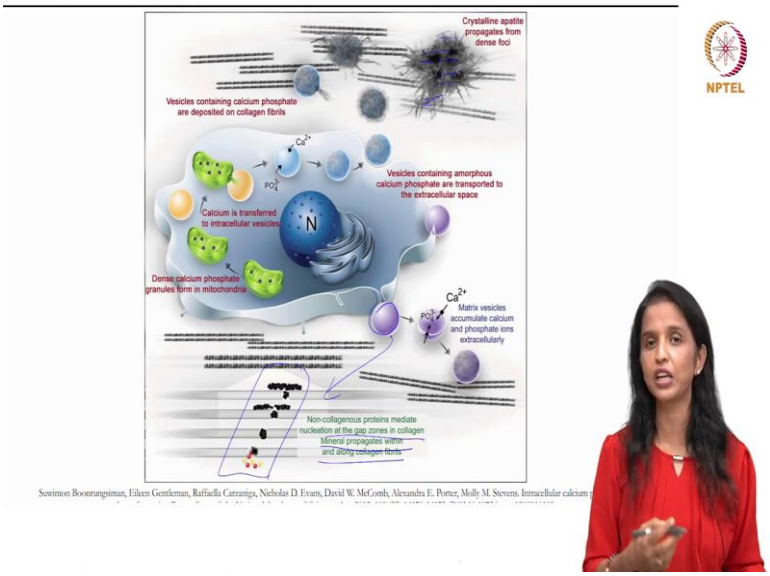

So, this particular picture is a very beautiful representation of the Matrix Vesicle Theory which is the most accepted theory of mineralization. So, you can find that this bigger cell is the osteoblast and then we can see that the matrix vesicles are getting budding, butted out from the synthetic cell and this actually contains vesicles contains amorphous calcium phosphate into the extracellular space and this further accumulates calcium and phosphates and this actually gets secreted.

We know that there are non-collagenous protein, the NCPs mediate the nucleation and the gap zones of the collagen. Mineral propagates within along the collagen fibers which we already saw in the collagen template theory but do understand collagen template theory is definitely accepted, only thing is the initiation comes from the matrix vesicles which is actually taking care of the mineralization of the collagen fibers and then we actually see that the vesicles containing calcium phosphates are deposited on the collagen fibers and the crystalline appetite propagates to form dense foci in this area.

So, this schematic diagram beautifully explains about how the dense calcium phosphate granules form in the mitochondria and the calcium is transferred into the intracellular vesicles and vesicles containing the amorphous calcium phosphates are transported to the

extracellular space and then it is moved away and then further accumulates calcium and phosphate extracellularly and that gets into the inter fibrillar spaces of the collagen fibers which then strengthens and then mineralizes and then solidifies as a hole.

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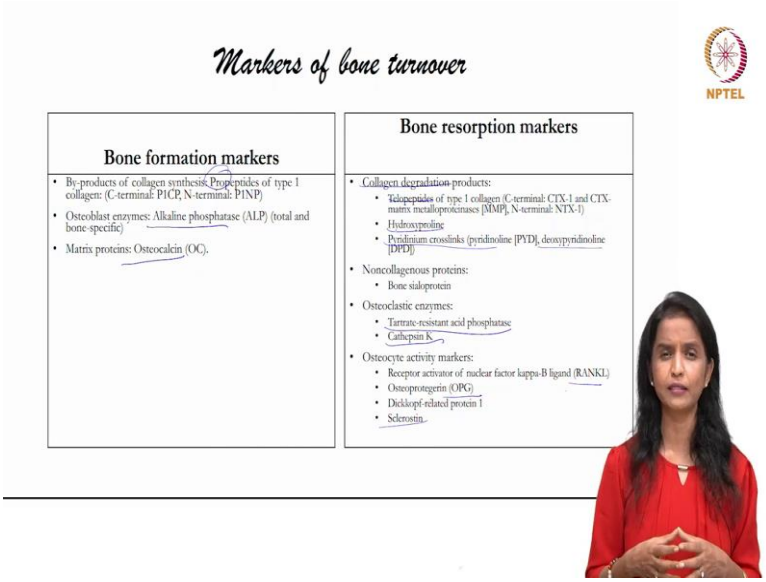

And this is again a very important thing to be studied. The moment we read about mineralization, we all know that mineralization can always be mostly physiological and pathological also. So, we need to know about what are the markers which can actually indicate these processes.

So, bone formation markers. So, if there is a bone formation happening what are the markers which are actually indicating the event of bone formation? So, those markers are your propeptides which are formative molecules of your type 1 collagen. We know that type 1 collagen are your organic matrix which are going to get mineralized.

So, the propeptides in that particular collagen byproducts or the initiator molecules of the collagen or the beginning molecules of the collagen are the ones which are very very indicative bone formative markers. Then we have alkaline phosphates which we already know which actually are secreted by the osteoblast. So, bone formation is by the osteoblast. So, the osteoblast is very well known to secrete ample amounts of alkaline phosphatase.

So, alkaline phosphatase is a reliable marker but nowadays propeptide evaluation, propeptide collagen evaluation is more reliable than even alkaline phosphatase or more sensitive form bone marker and then we have the mass matrix protein that is osteocalcin which is considered as a bone formative marker.

We have a bigger list for bone resorption markers here. So, the first is collagen degradation products and that if you see here it is Propeptides that is in the formative side, but as the collagen which is formed, a mature collagen is breaking down into smaller events. So, there it is called as telopeptides and then the breakdown products will also have hydroxyproline, pyridinium crosslinks, deoxypyridinoline are all breakdown products of mature collagen fibers.

Whereas here in before we have formative bone formative markers that those are indicated by the propeptides whereas, if a mature collagen fiber is going to break down it would release telopeptides, hydroxyprolines and pyridinolines which can be detected and then they would be indicative of bone resorptive process.

We also have non collagenous protein that is bone sialoprotein which are bone resorptive markers or markers which are suggestive of bone resorption and then we also have a very important enzyme here that is acid phosphatase, that is tartrate resistant acid phosphatase which we already saw the trap and Cathepsin which is again a very important marker of osteoclastic activity and then further we saw about osteoblast and osteoclast.

We also should know whether there is anything specific about osteocyte marker. So, we have the RANKL, the OPG and we have the Dickkopf related protein one and the Sclerostin marker which is actually indicative of bone resorptive activity. These are specific to osteocyte. So, some are specific to the osteoclast and some are specific to the osteosite.

So, the by with this markers of bone turnover we are actually completing learning of mineralization mechanics which are very important in oral biology. Because this particular topic forms the basis of the physiological mechanisms and the pathological mechanisms happening in the orofacial region.

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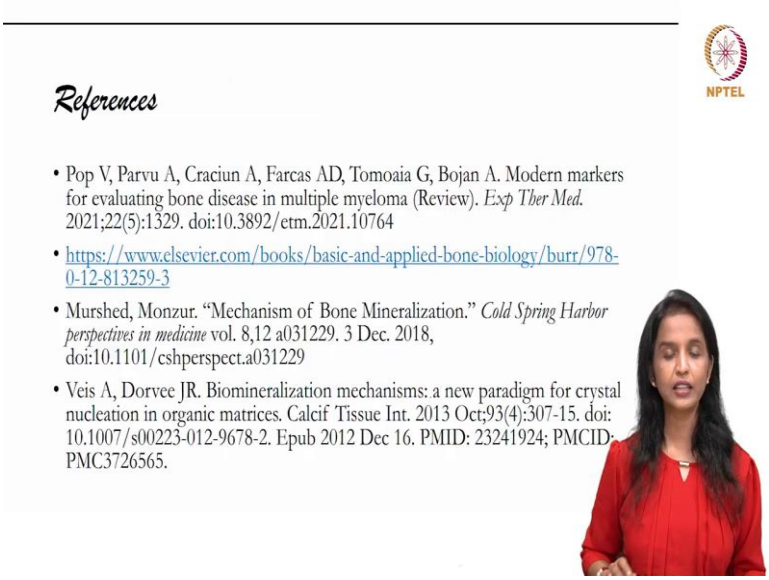

Thank you for your patient listening and these are the references which can be read to know more about mineralization dynamics.