Tissue Engineering
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Lecture – 30
Skin Tissue Engineering Part 1

Hello, everybody. Today, we are going to talk about Skin Tissue Engineering.

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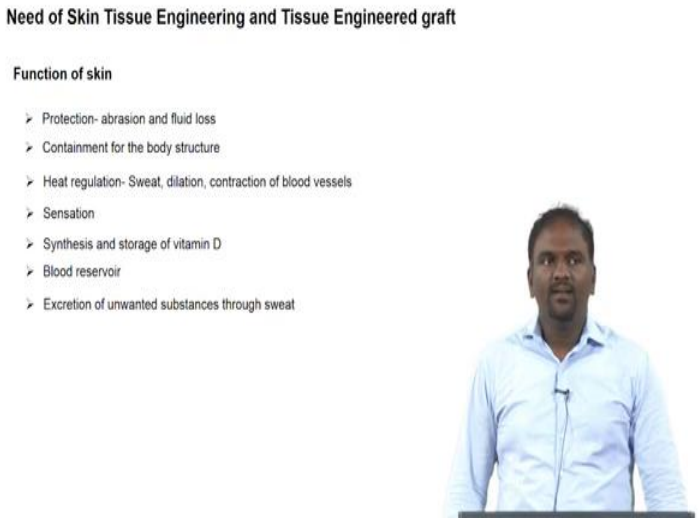

Before going into that skin tissue engineering or tissue-engineered graft, we should need to know about the function of the skin. As we all know that skin is the largest organ of the body, and its main function is protection from the environment, especially abrasion and fluid loss. It gives the containment for the body structure and also the organs present inside the body.

It also involves in heat regulation like in the form of sweat, dilation, contraction, and blood vessels. It also involves in the sensation of nerves like while we touch that conduction of the things will be passing into and will be sensed. It also involves in the synthesis and storage of vitamin D. Further, it also acts as a blood reservoir. It also plays an important role in excreting the unwanted substances through sweat.

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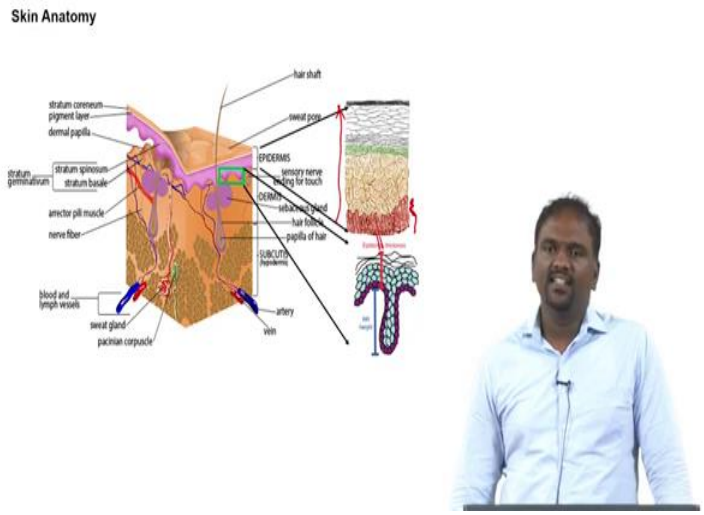

Before developing any skin graft or something, we need to know about the anatomy of the skin. If we see the skin, it has two layers: the first layer is dermis, and the second top layer is epidermis. The lower bottom layer is not part of the skin, but it also plays a role while we discuss about the skin. That bottom layer is subcutaneous tissue, or it is called as hypodermis. It has many parts. Attached to the hair follicles, we have a sebaceous gland. The Sebaceous gland produces sebum. That sebum gives lubrication for that hair follicle, and this is also responsible for making the skin waterproof. And, it also acts as a primary defense system.

Then attached to that sebaceous gland, we have arrector pili muscle; that arrector pili muscle is responsible for that pulling of hair when goosebumps happen. We have the nerve conducts. So, this is responsible for the sensation, and we have blood vessels for the transport of nutrients and other things from the subcutaneous part to the dermis and further dermis to the epidermis part.

We have epidermis. So, epidermis has various layers; the first layer is epithelial cells. So, this first layer contains epithelial cells, and this is called stratum basale. Then because of the blood vessels, whatever is coming from the dermis part, the blood supply is going into that epidermis, and the cells will keep dividing, so that division of the cells goes in the top portion.

From the basale, that goes into that next layer that is called stratum spinosum. In stratum spinosum, the epithelial cells get keratinized, and apart from the keratinocytes, we have melanocytes that are responsible for the color of the skin. The next layer of that stratum spinosum is stratum granulosum. Here, the cells flattening happens, and over there, we have stratum corneum; that is a final layer of the epidermis, and that acts as the shed of the old cells.

We have in-between layer epidermis, and dermis is the junctioned epidermal-dermal junction. This is called rete ridges. These rete ridges play the main role in giving mechanical strength to the epidermis, and it also gives adherence to the dermis and epidermis because of this epidermal junction. Apart from that, the transfer of soluble molecules from the dermis to the epidermis is happening through this junction.

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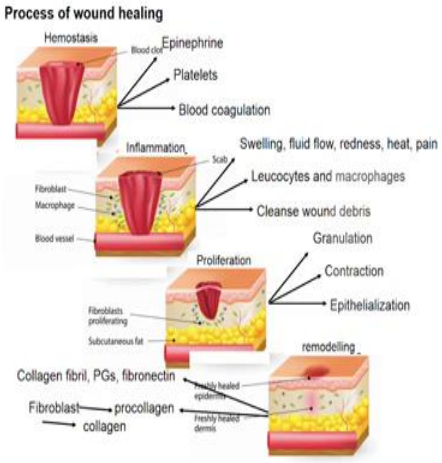

When there is a wound in normal skin, what happens is by innate mechanism, the wound will get healed. It involves a series of steps. The first step is hemostasis. In hemostasis, what happens is the blood coagulation will occur. During that blood coagulation, at the wound site, epinephrine will be released. Once that epinephrine is released, the platelet coagulation will start, and the complete process is called hemostasis.

Then the next step to hemostasis is inflammation. Once the blood started coagulating, the swelling and other things will happen because of the inflammation. During swelling, it also produces heat, and fluid flow also will be more, and obviously, the pain also will be

getting. Then herein we have the defense system also will play an important role. When there is a wound, the foreign particles will try to invade into the body. That time that leukocytes and macrophages will start developed into that particular site. The first site of the mechanism is by leucocytes. The leucocytes will be replaced by the macrophages. So, macrophages will clean that debris of the wound.

Then the next step is proliferation. Proliferation is mainly by fibroblast, present in the dermis. If the wound is happening only in the epidermis part, that proliferation will happen with the help of keratinocytes. This involves three different types or like classification.

The first one is granulation. In the granulation, what happens is the cells will start dividing. If we take fibroblasts or dermis as the wound site, so, the fibroblasts will start doubling, and it will be creating multiple cells, and after that, once that cells start dividing; obviously, the size of the things will come down, and contraction will happen, then epithelialization will happen.

The next step is remodeling; in the remodeling, what happens is that fibroblasts will develop the extracellular matrix; that extracellular matrix contains collagen, fibril, proteoglycans, and other fibronectins. Fibroblasts initially will produce procollagen, that procollagen will twist together and that form strong collagen. Many collagen molecules will come together, and they form a very strong network. Because of this network only, we get the fresh or regenerated tissue.

If this collagen is not twisted and it is not forming a proper network, what happens will be that mechanical strength of the tissue whatever is formed will not be equivalent to the normal skin. If we get a wound, we ourselves will be able to see such kind of difference.

When that tissue starts forming, that strength of the tissue will be very smooth. Once that new tissue is formed, we will be able to see that the strength of the tissue will be equivalent to the earlier tissue. The reason is that collagen remodeling is what happens at that site.

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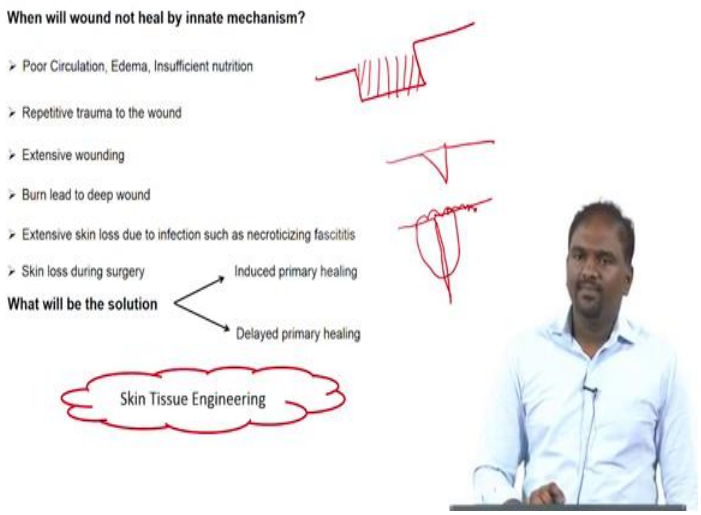

This is a normal mechanism of wound healing. In a few conditions, this normal mechanism will not happen. One such condition is poor circulation. When there is poor circulation of blood into that wound site that normal wound healing will not happen. When there is edema or insufficient nutrients or repetitive trauma to the wound, or an extensive wounding, then the normal wound healing process will not happen. Burn leads to deep wound; if there is a deep wound, it is very difficult for that wound to heal. Then, the next reason could be extensive skin loss due to infection such as necrotizing fasciitis. During surgery, also we get skin loss. So, that time also it is very difficult for that skin to regenerate and wound to heal.

When the innate mechanism is not happening, the probable solution could be induced primary healing. Consider we have wound here like this. So, in induced primary healing, what we do is this portion, and this portion will be combined together like this way, and they put some stitches over here. So, that will be totally covered. When this is happening, we will be able to heal, but when that wound bed size is more, it is very difficult to bring both the sides of the wound and to bring that down into that portion.

Then the second step is delayed primary healing. In the delayed primary healing, only we will be talking about that skin graft or tissue-engineered based product. In delayed primary healing, what happens is, consider we have wound like this. Both the skin cannot be brought together, and we cannot stitch it. For making that fibroblast to grow,

we need to keep something over here. So, that will be achieved by the graft whatever we are developing.

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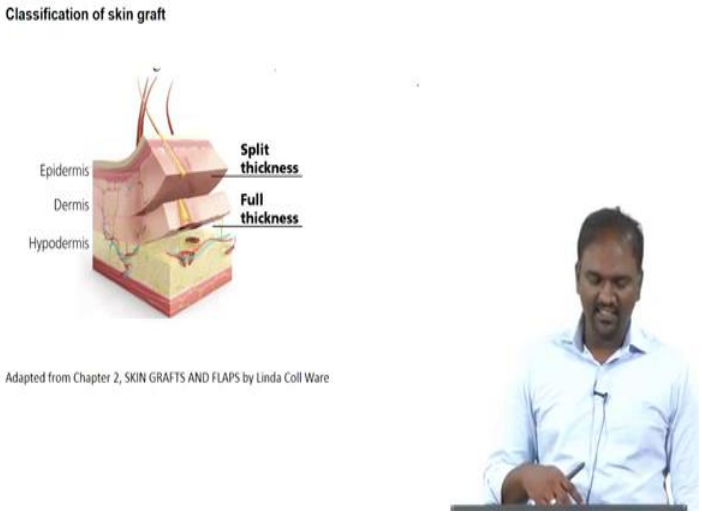

Classification of the skin involves two grafts; the first one is split-thickness graft, and the second one is full-thickness graft. Split thickness graft involves only the epidermis regeneration; full-thickness graft involves the epidermis together with the dermis regeneration. As I told in that anatomy part, the hypodermis is not part of the skin, but it plays a role when we discuss about tissue engineering.

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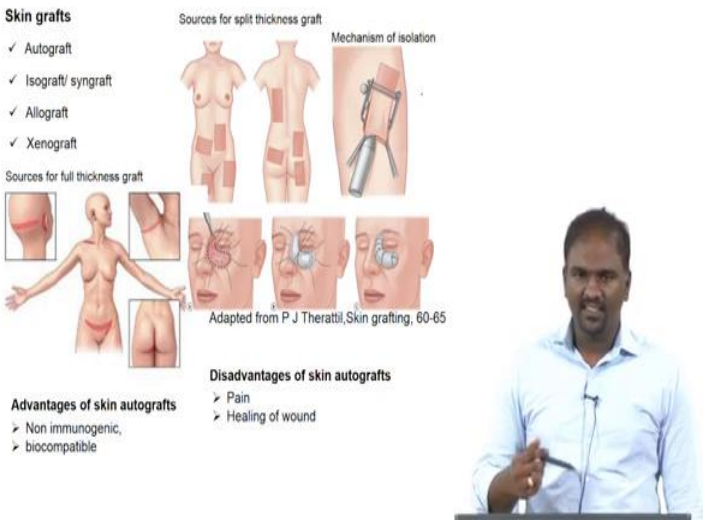

In a skin graft, we have autograft. Autograft is from the own patient sample; they will be taking the tissue, and they will be placing it. In Isograft, the genetically equivalent sample will be taken from the same species; the graft will be taken. In the xenograft from one species to another species, it will be transferred. Among this, an autograft is advantageous because of various reasons.

The sources for a split-thickness graft could be like the abdomen or thighs or backside of the buttocks. As I told that split-thickness graft involves only that epidermis part. In the epidermis part, what they do is they use a dermatome, took at that splice. So, that splice will be further what they do is they make mesh into it, and they will be placing into that wound site.

Then we have sources for the full-thickness graft. In the full-thickness graft, what they do is they will cut the graft from the backside of the head and buttocks and also armpits. From these portions, they can get the full-thickness graft. A full-thickness graft is like excision of that complete part of that skin. It is not like we do not need to use any dermatome or any other specialized instrument to cut that splice whereas, that complete skin will be taken. They will be excising that portion, and they will be placing into that place wherever it is required.

When they do the surgery like after cutting the portion from the particular part, they will give some pressure onto that place, and they will be placing support like this way. So, this is called a bolster. They will keep the bolster, and they will stitch it. So, the reason for doing such a thing is if you do not keep something over that portion wherever that graft is placed, that will not be in that position for a long time. For making that sure, they are keeping some bolsters into that surgery wherever they have done, and they will be covering it by stitching.

The advantage of these skin grafts are like it is taken from their own body. So, it is biocompatible, and it is non-immunogenic. It also has disadvantages. So, the main disadvantage is that it is taken from their own body, so, obviously, it will give pain to the patient, and then another thing is it will again create a secondary wound. That secondary wound healing will be again a problem. So, these are all the drawbacks whichever we get from the autograft-based skin graft.

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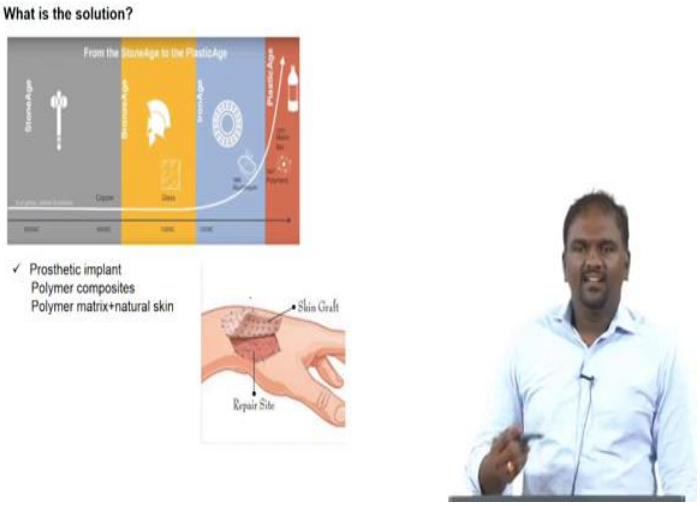

What could be the solution means like have come across from stone age to now we are in the plastic. In the plastic age, we are using multiple polymers. We can use polymers for various good applications like these kinds of things. We can make some prosthetic implants made with the polymer. Instead of that graft taken from the patient's body, we can use the polymer as a skin graft because ultimately, we are developing the graft just to cover that wound bed. Once we covered it and that graft is completely biocompatible, the cells will start infiltrating into it, and it will try to form the normal structure of the skin.

So, this is how that will work. Consider this is our skin graft, and that skin graft is placed into that wound bed. Once it is placed and it will be stitched, or they will be doing some dressing, then once that dressing is completed; obviously, that wound healing will occur, and that will form the normal skin.

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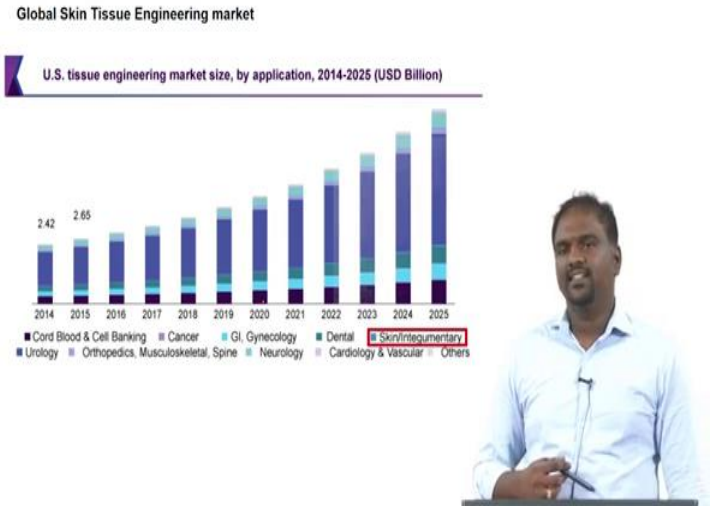

In the global tissue engineering market, the highest market is based on the skin.

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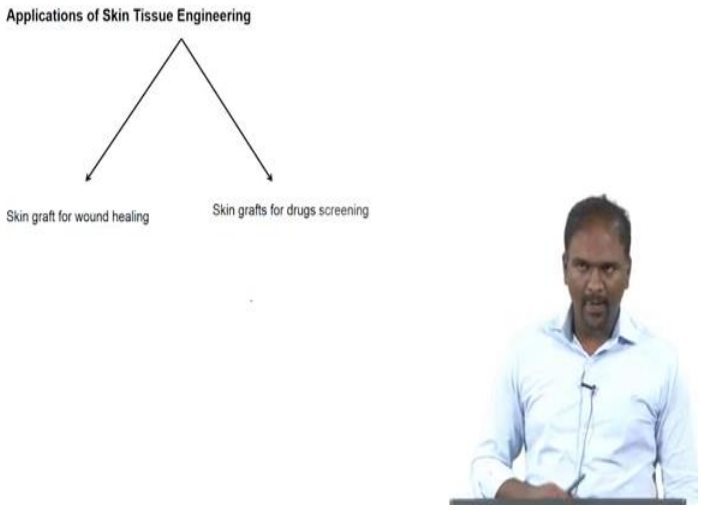

Once we develop that skin graft, so that can be used for two different applications. The first application could be that skin graft can be used for wound healing. As I told the wound whichever cannot be healed at by innate wound mechanism, that type of thing needs some graft. For such kind of application, you can use this skin graft.

Another type of application is for cosmetics-related things. We are developing so many drugs and molecules. So, for checking or screening that drug molecule, we are using so

much of animals and humans. That involves ethical considerations and many problems. If we are able to develop a skin graft equivalent to the normal skin, so that skin graft itself can be used for drug screening implications.

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Hello, everyone. I am Vasudha, an MS student in Doctor Vignesh Muthuvijayan's lab. Today, we are going to discuss various design strategies that have been used to synthesize tissue-engineered skin grafts. Artificial skin is designed in two stages – stage 1 and 2. Stage 1 applies to short term acute use, while stage 2 applies to long term chronic use. Stage 1 deals with the physical and mechanical aspects of the design, while stage 2 deals with biochemical aspects.

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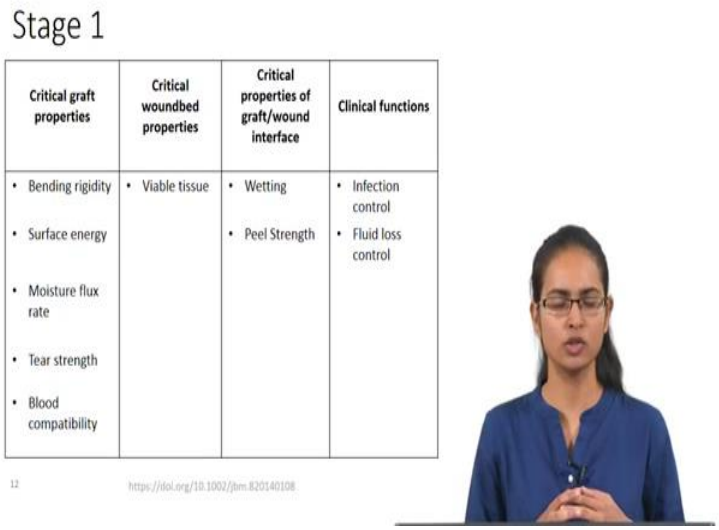

In stage 1, the major critical graft properties that are considered include bending rigidity, surface energy, which affects the wetting of the graft, moisture flux rate through the graft, tear strength, and blood compatibility. The critical wound bed properties such as viable tissue are of considerable importance, and the critical properties of the graft wound interface include wetting and the peel strength. And, the clinical functions that are most important at this stage are infections and fluid loss control.

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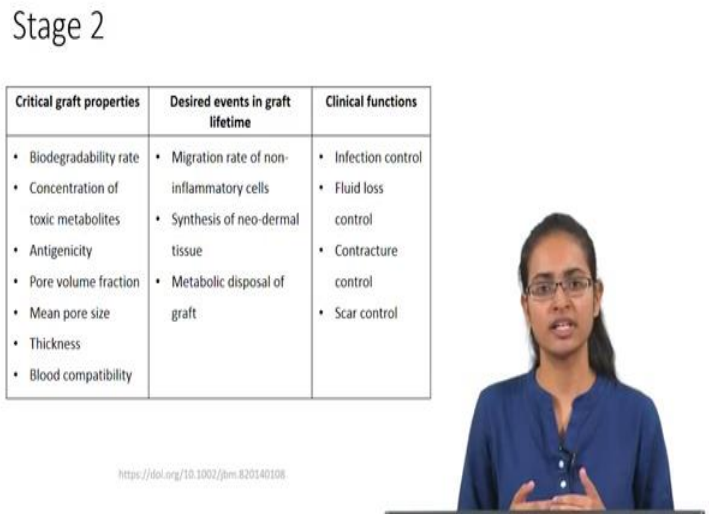

In stage 2, the graft properties that are important are biodegradability, and the non-antigenicity of the membrane is crucial. Also, the porosity of the membrane, the pore size of the membrane, the thickness, and the blood compatibility. The desired events in the graft lifetime include migration of non-inflammatory cells such as fibroblasts and keratinocytes, a synthesis of neo-dermal tissue, and also the metabolic disposal of the graft. So, in this stage, other than the infection and fluid loss control, contracture and scar control are considerably important.

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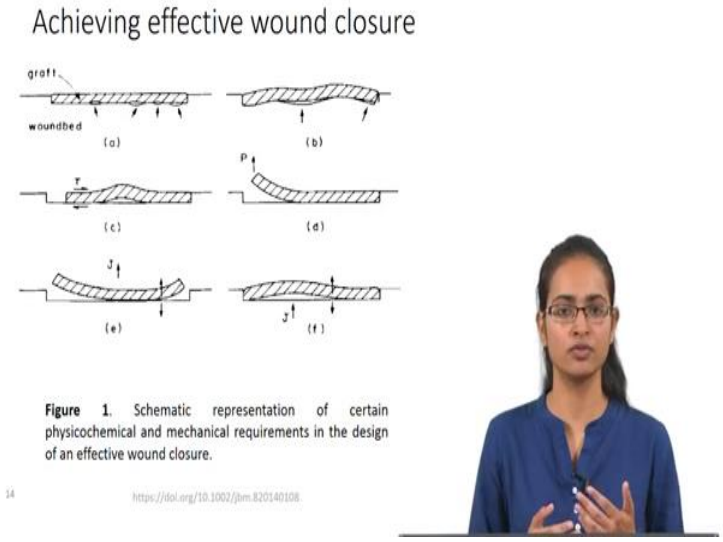

There are different physicochemical and mechanical aspects that are important for achieving effective wound closure. In this representation, you can see the formation of air pockets because of ineffective wound closure.

In figure b, you can see that due to excessive flexural rigidity of the graft, there is the formation of air pockets between the at the wound and graft interface. In figure c, due to excessive shear stresses, there is the formation of air pockets. In figure d, that is due to peeling, excessive peeling forces, and then figure e, due to excessive moisture flux rate through the graft, there is dehydration of the membrane at the corners, which causes lifting of the membrane. In figure f, the moisture flux rate is insufficient, due to that, there is excessive fluid accumulation, which can cause edema. So, all these should be prevented to achieve effective wound closure.

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What is the long-range design objective for artificial skin? You should design a membrane that provides satisfactory wound closure to prevent infection and fluid loss. The graft or the membrane should be solid, degradable, and non-antigenic, and also it should provide an early opportunity for cell migration and connective tissue synthesis; that is collagen deposition inside the membrane or graft.

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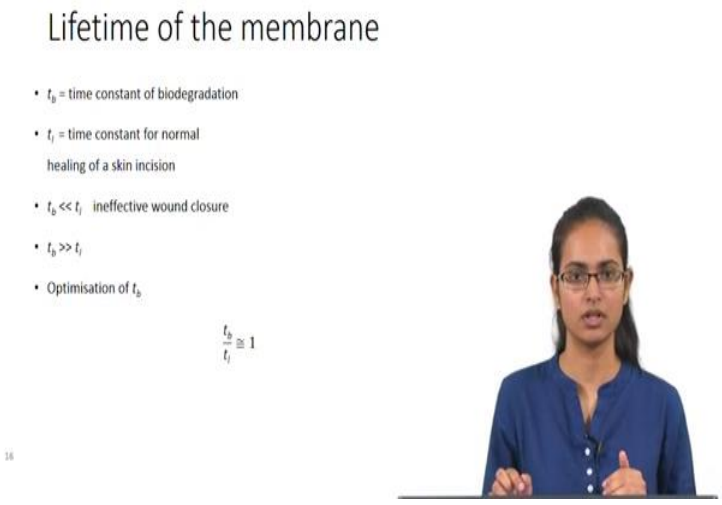

The lifetime of the membrane is a very important aspect. Here, t_b is the time constant of biodegradation, and t_l is the time constant for normal healing of the skin incision. The t_b

that is the biodegradation rate has to be optimized. Because if the t_b is much lesser than t_l, then there is ineffective wound closure; that is the membrane is getting degraded before the healing process takes place. Also, if it is much higher than t_l then it has been observed that there is fibroid tissue formation under the graft.

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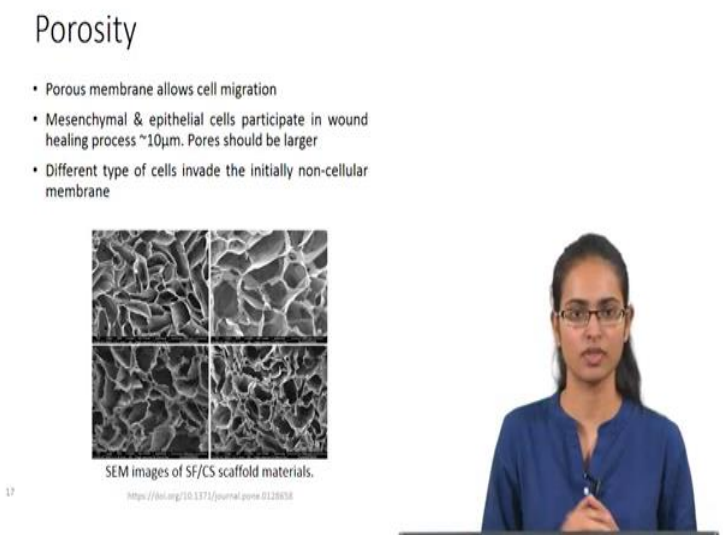

The next aspect is the porosity. Porosity is crucial to allow cellular migration into the graft and also important for the exchange of gases and nutrients. Porosity is also important to allow the cells that participate in the wound healing process to migrate into the graft; majorly being mesenchymal and epithelial cells. These cells have a size of around approximately 10 micrometers.

So, the pore size of the membranes should be larger than this to allow cellular migration. Also, the different types of cells invade the initially noncellular membrane. This is a scanning electron microscope image of a silk fibroin/chitosan blend scaffold. So, you can see the interconnected pores in the image.

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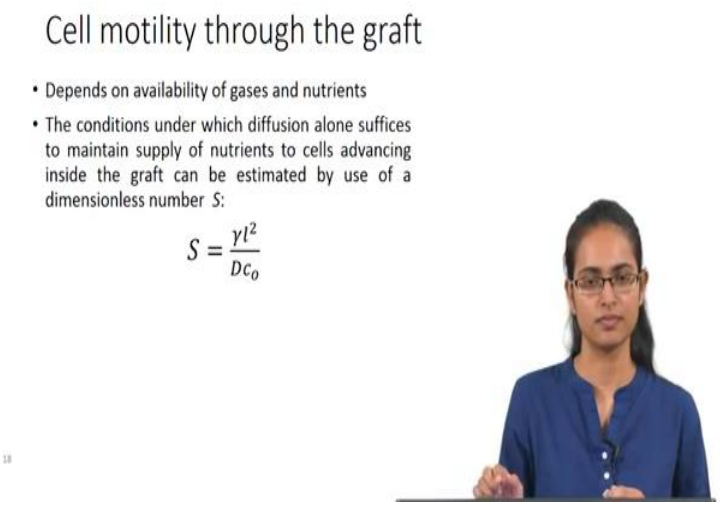

Cell motility through the graft depends on the availability of gases and nutrients. So, the conditions under which diffusion alone suffices to maintain the supply of nutrients to the cells that are advancing through the graft is estimated using a dimensionless number S.

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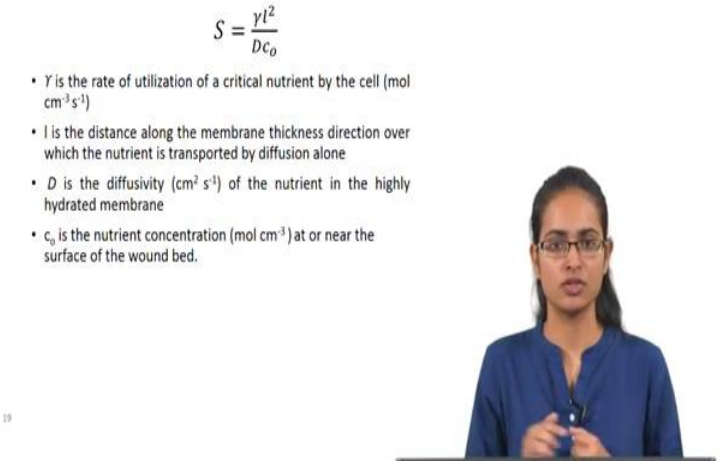

In this equation, γ is the rate of utilization of a critical nutrient by the cell; l is membrane thickness; distance along the membrane thickness along which the cell has to migrate itself; D is the diffusivity of the nutrient in the membrane; c_0 is the nutrient concentration at or near the surface of the wound bed.

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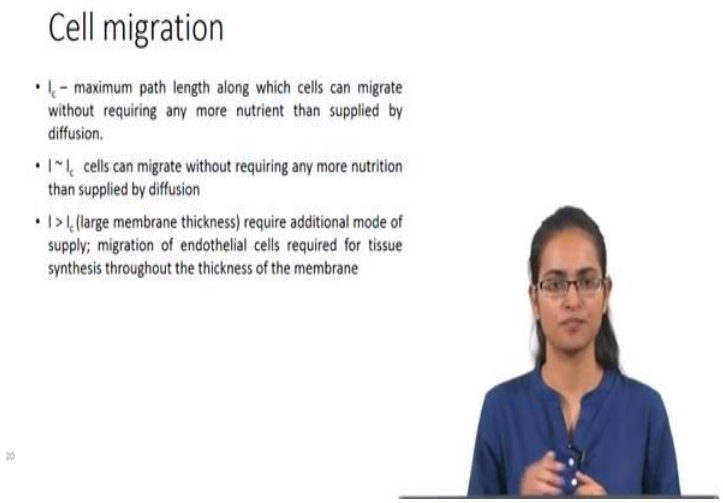

l_c is the maximum path length along which cells can migrate without requiring any more nutrient than supplied by diffusion. So, if your membrane thickness l is approximately equal to l_c, then the cells can migrate without requiring nutrients other than that supplied by diffusion. However, if the membrane thickness is much larger, then what happens is you need an additional mode of nutrient supply. Then there is a need for migration of endothelial cells and vascularization, which is an important aspect of skin tissue engineering.

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Endothelial cells have a migration rate of around 0.4 mm/day, and fibroblasts cells around 0.2 mm/day. This rate is usually insufficient to cover the surface of the graft in a period comparable to wound healing. An alternative approach that is used is to culture epithelial cells on the membrane surface at an appropriate time following grafting.

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The materials used for skin tissue engineering have to satisfy certain needs; providing a barrier layer of renewable keratinocytes and securely attach to the underlying dermis, promote vascularization, and also provide elastic structural support for the skin.

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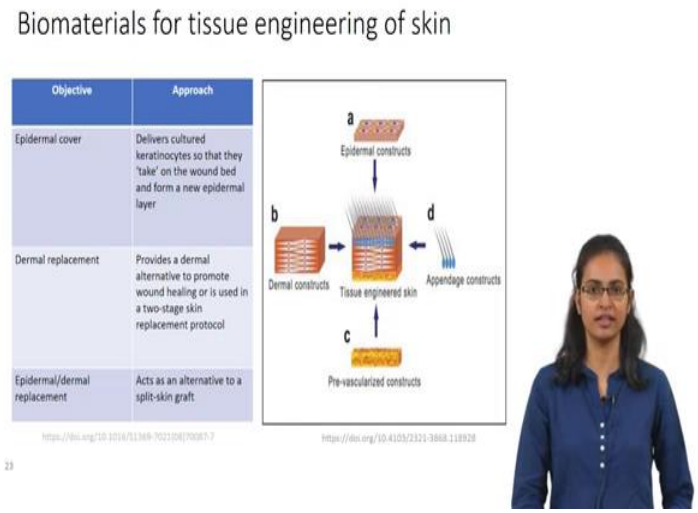

The biomaterials used for skin tissue engineering can be an epidermal cover which can deliver cultured keratinocytes. This is the upper thin layer of the skin. This usually is involved in the prevention of infection and fluid loss, or it can also be a replacement of the dermis, which is the underlying thicker layer. So, this layer is mostly composed of fibroblasts. Or it can also be an epidermal and dermal replacement, which is usually split skin graft.

Also, the tissue-engineered skin can contain appendage constructs such as hair follicles and sweat glands; the presence of these helps us mimic the natural skin more closely. Also, the pre-vascularized constructs can be provided to supply nutrients to the graft, like we discussed if the thickness of the graft is much higher than diffusion alone is not sufficient to provide gases and nutrients.

In the next lecture, we will look at different materials that can be used for skin tissue engineering, and we will also look at various fabrication techniques that are used to synthesize tissue-engineered skin grafts.