Tissue Engineering
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Lecture - 31
Skin Tissue Engineering Part 2

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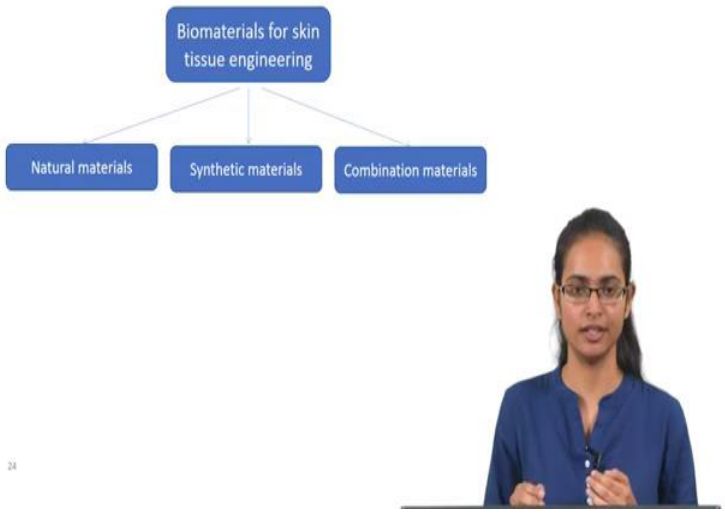

From here on, we will discuss the major components of the Tissue Engineering Triad that are the materials, the cells, and the signals required for skin tissue engineering. Biomaterials that are used for skin tissue engineering can be classified as natural, synthetic, and the combination of the two. So, we will discuss each one of them in detail.

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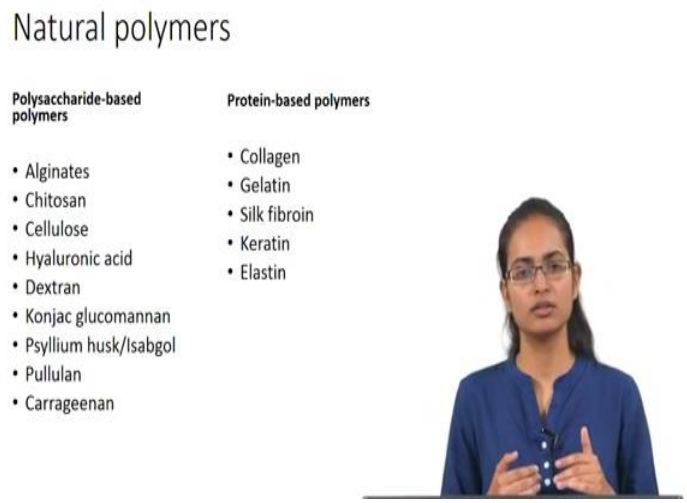

Natural polymers that are used for skin tissue engineering can be polysaccharide-based polymers or protein-based polymers. These are some examples of natural polymers that are used for skin tissue engineering; this is however, not an exhaustive list. So, alginates, chitosan, cellulose, hyaluronic acid, dextran, pullulan, carrageenan, konjac glucomannan, and isabgol are newer polymers that are being explored for skin tissue engineering. Protein-based polymers such as collagen, gelatin, silk fibroin, keratin, and elastin are being explored for skin tissue engineering; we will look at each one of them in detail.

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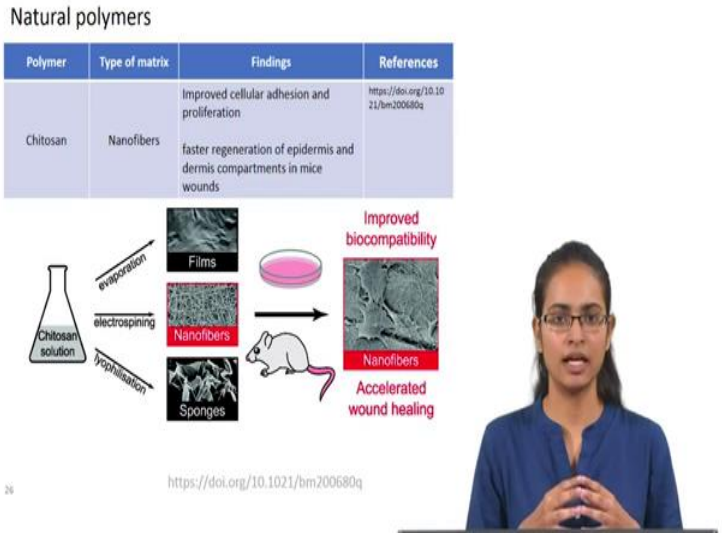

In natural polymers, chitosan is a very popular material used for skin tissue engineering; obtained from chitin, which is found in the fungal cell wall, and also the exoskeleton of insects. Chitosan can be processed into different types of matrices, such as films, nanofibers, and sponges. So, several studies have been done using chitosan. Here it shows an in vivo study where chitosan has been shown, it is highly biocompatible and has been shown to accelerate wound healing, and also chitosan has helped improve cellular adhesion and proliferation.

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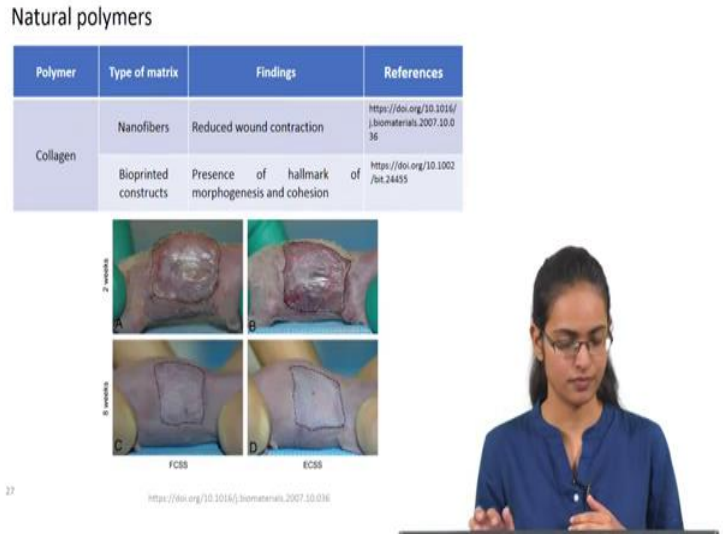

Collagen is a protein-based polymer. It is the major portion of the extracellular matrix, the ECM; collagen also can be processed as nanofibers or 3D printed constructs. Here you can see that using a collagen skin substitute, an acceleration in wound healing has been achieved; in both freeze-dried skin substitutes and electrospun skin substitutes. Reduced wound contraction and also a presence of hallmark of morphogenesis and cohesion, which shows the formation of the natural skin tissue has been observed.

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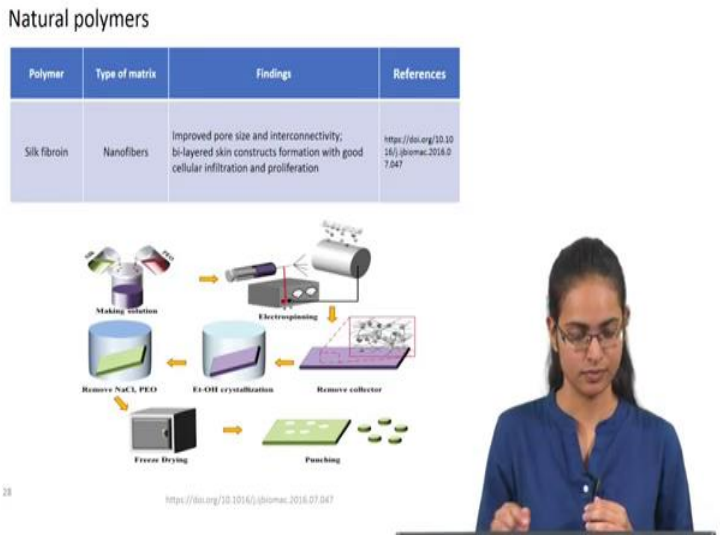

Silk fibroin is another natural polymer; here, it has been processed into nanofibers using electrospinning. Electrospun silk fibroin has shown improved pore size and interconnectivity. The improved pore size and interconnectivity helps in good cellular infiltration and proliferation.

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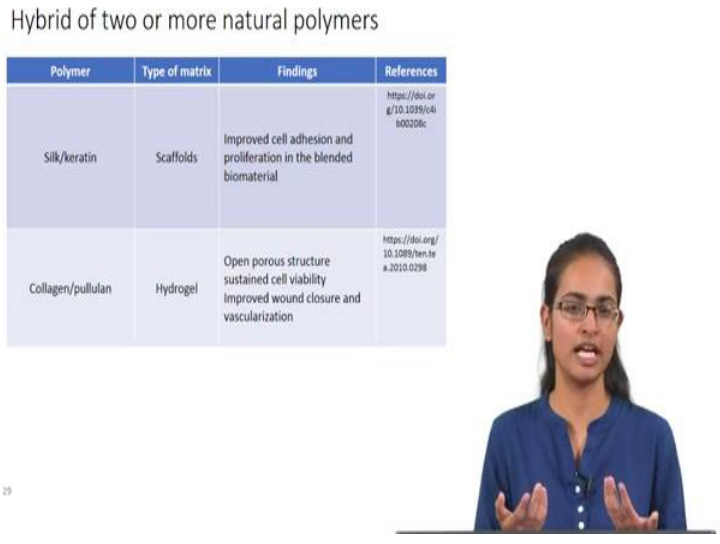

Next stand-alone natural polymers, hybrid of two or more natural polymers, have also been used; such as silk/keratin scaffolds. Here, the keratin component, which contains

RGD sequences, helps in improved cellular adhesion and proliferation than compared to having silk fibroin scaffolds alone. The blended material showed superior properties.

Also, collagen/pullulan hydrogels have been synthesized. Collagen is usually used to make hard biomaterials to make softer biomaterials. It is often blended with other natural polymers to make hydrogel systems. So, pullulan is one such example. In this hydrogel, they obtained an open porous structure with improved cell viability and also achieved improved wound closure and vascularization.

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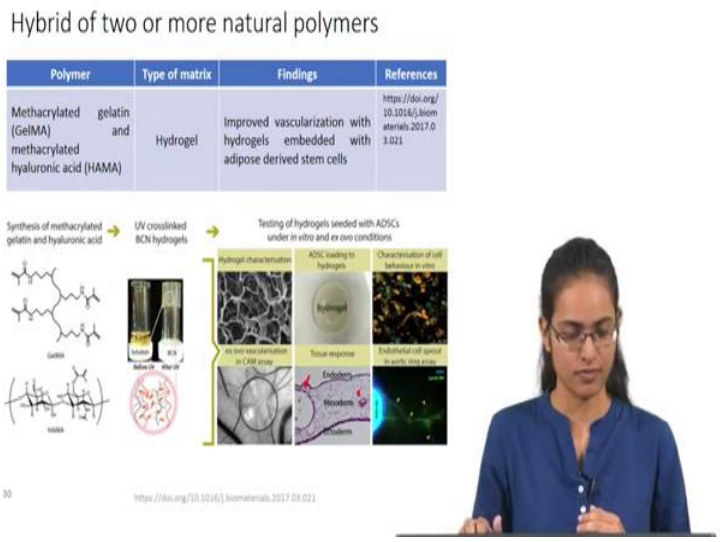

Methacrylated gelatin and methacrylated hyaluronic acid have been blended, both natural polymers. Hyaluronic acid is also an ECM component; gelatin is a natural protein-based polymer. Both the polymers have their own disadvantages, such as hyaluronic acid does not help with cell adhesion, and gelatin is poor in it is mechanical properties.

So, the blend of the two can help us overcome the shortcomings, and also physical or chemical modification of naturally occurring polymers can help improve their properties. Here in this example, the blend has been processed into a hydrogel, and they have embedded adipose-derived stem cells into the material for improved vascularization.

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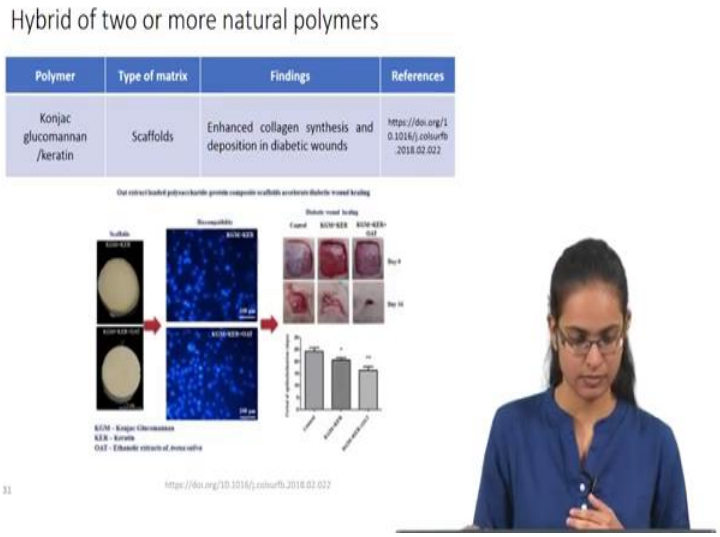

Konjac glucomannan/keratin. Here, konjac glucomannan is a polysaccharide-based polymer, and keratin is a protein-based polymer. The two have been blended to form scaffolds. In these scaffolds, they have done an in vivo study where it showed enhanced collagen synthesis and deposition in diabetic wounds, and also, the scaffolds have been shown to be highly biocompatible.

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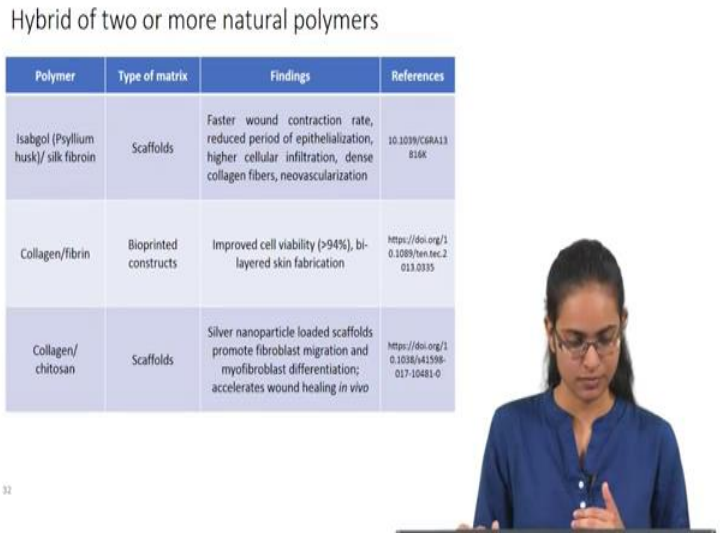

Also, other natural polymers have been blended together to have superior properties as skin tissue-engineered products, such as isabgol/silk fibroin, collagen/fibrin, bioprinted constructs, or collagen/chitosan scaffolds.

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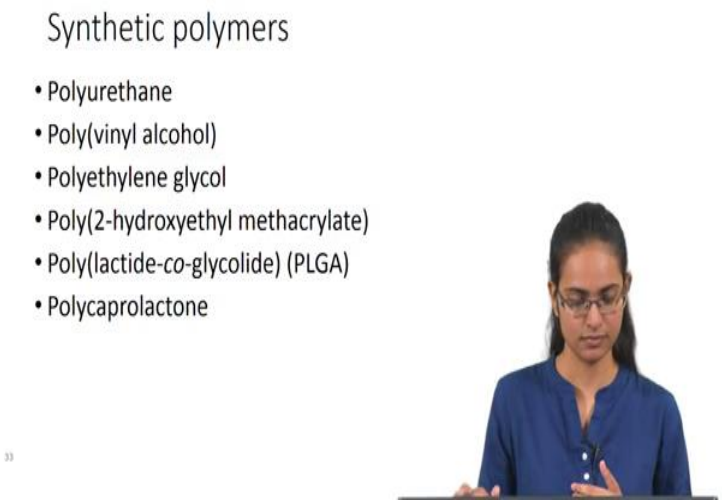

Now we look at synthetic polymers, which have also been used for skin tissue engineering; polyurethane, PVA that is Polyvinyl Alcohol, PEG-Polyethylene Glycol, poly HEMA, polyhydroxy ethyl methacrylate and PLGA Poly lactic-co-glycolic acid and polycaprolactone.

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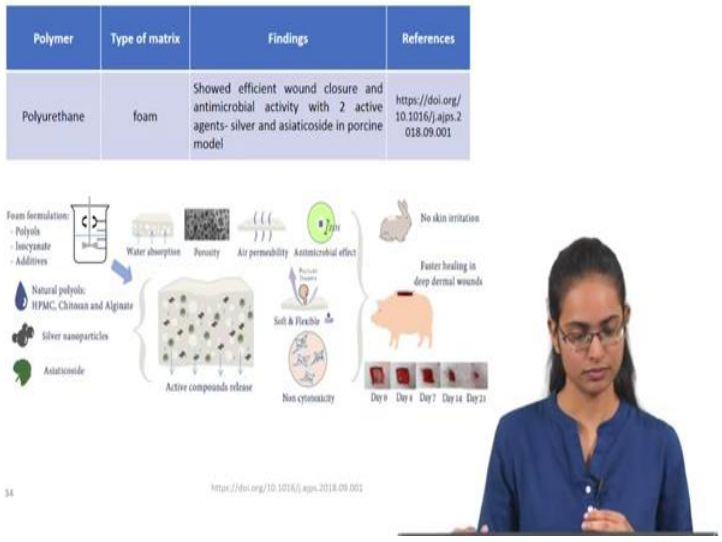

Polyurethane is a very popular synthetic polymer used for skin tissue engineering; several commercially available wound dressings are there, which are made from polyurethane.

This is an example of polyurethane foam, also you can load different bioactive agents into the materials to render better wound healing properties. Here they have blended; they have added silver and asiaticoside into the polyurethane material. They tested the effect of wound closure in a porcine model. Here, the material has shown to be biocompatible as there is no skin irritation, and also they have observed faster-wound healing in deep dermal wounds.

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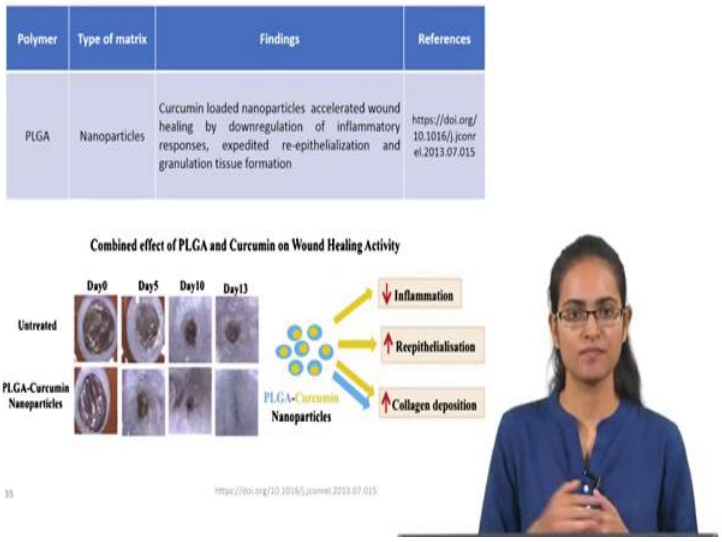

PLGA is a copolymer of lactic acid and glycolic acid. Here in this example, they have a synthesized nanoparticle, and they have loaded curcumin, a bioactive agent. So, they have observed reduced inflammation, increased re-epithelialization, and increased collagen deposition, which are very important for wound healing.

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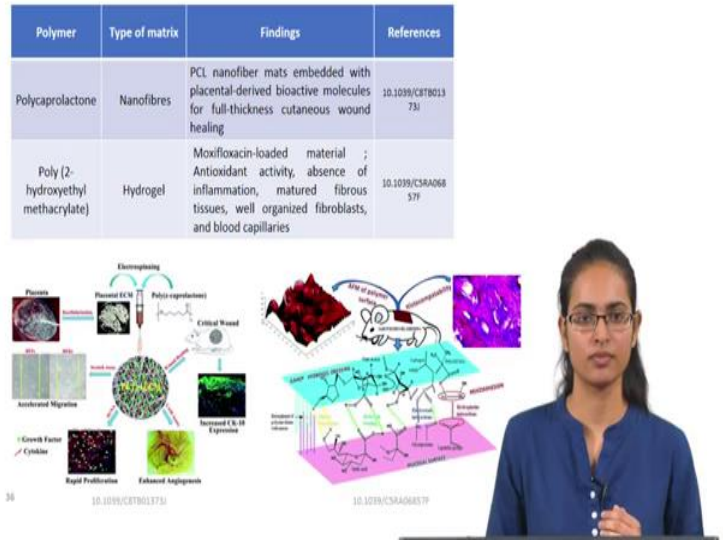

Polycaprolactone is another polymer. Here, they have synthesized them as nanofibers using electrospinning. They have been embedded with placental derived bioactive molecules for wound healing. Poly HEMA is another synthetic polymer. They have synthesized it as a hydrogel, loaded with moxifloxacin, and they have seen antioxidant activity and absence of inflammation.

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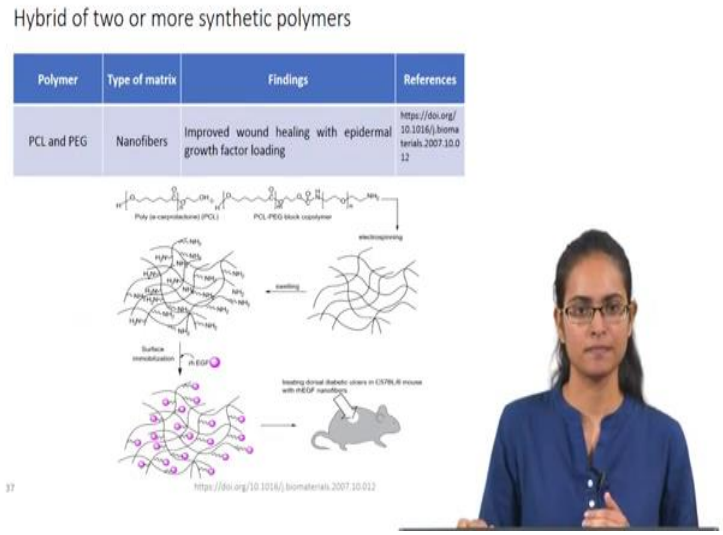

Like natural polymers, two or more synthetic polymers have been blended to achieve better properties. Such as PCL and PEG block copolymer that has been synthesized and

electrospun. Here, you can see the free amino groups to which they have attached epidermal growth factor, and have done in vivo studies to show improved wound healing with epidermal growth factor loading.

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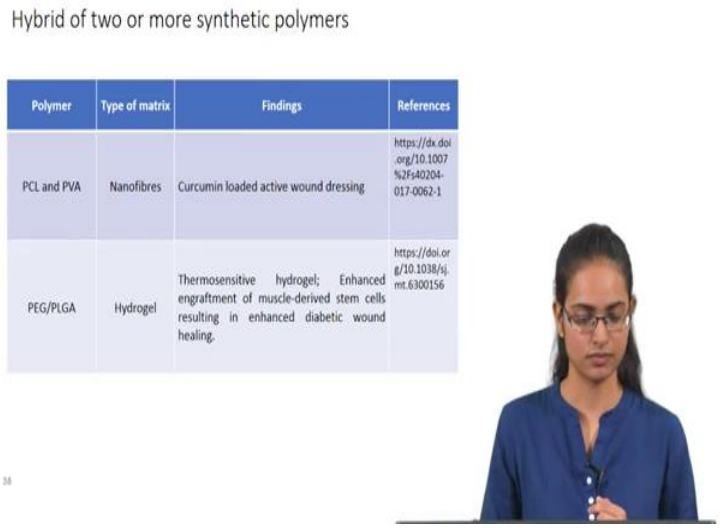

Also, there have been other synthetic polymer hybrids. Curcumin loaded PCL, and PVA nanofibers showed improved wound healing. PEG and PLGA hydrogel, this was synthesized as thermosensitive hydrogel or environmentally responsive hydrogel. This showed enhance engraftment of muscle-derived stem cells that were seeded on the grafts.

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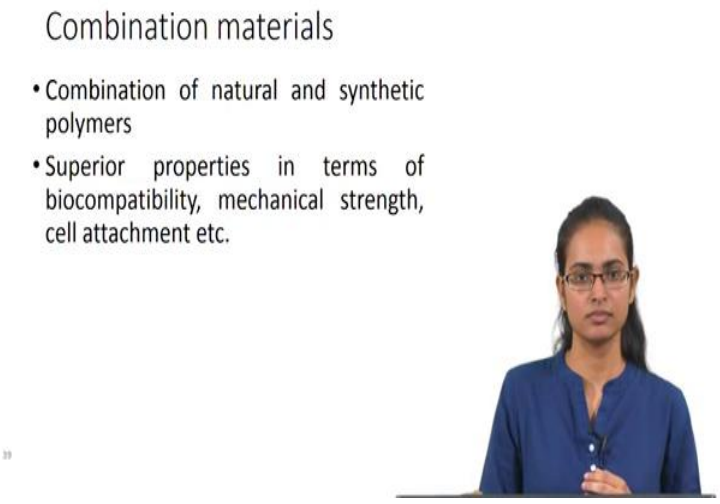

The combination of natural and synthetic polymers gives better properties for skin tissue engineering. Natural polymers can provide biocompatibility to the graft, while synthetic polymers can provide the required mechanical strength. Also, natural polymers help with cell attachment and adhesion and proliferation.

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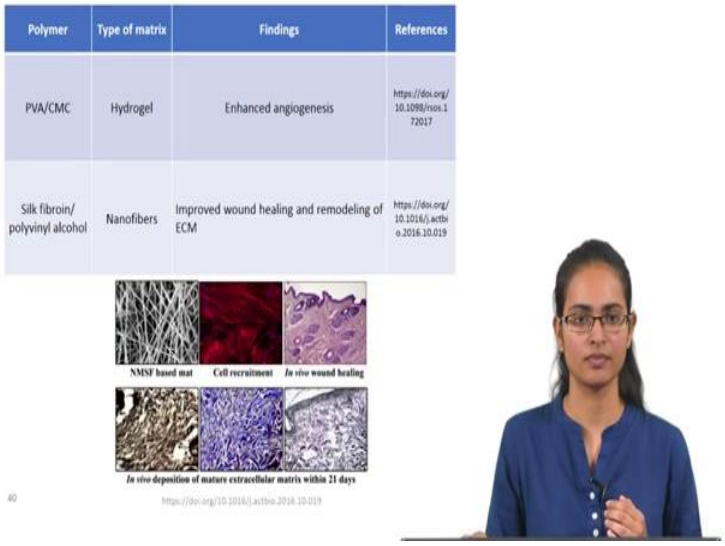

PVA/CMC is a blend of Polyvinyl Alcohol and Carboxy Methyl Cellulose, which is a carboxymethyl derivative of cellulose. Here, PVA is the synthetic component, and CMC is the natural polymer. PVA provides the mechanical properties to the material. PVA is, however, highly hydrophilic, and it does not help with cell adhesion. So, carboxymethyl cellulose can help with cellular attachment and proliferation. The blend has been synthesized as a hydrogel, and in this particular study, they have loaded reduced graphene oxide into the material and have found enhanced angiogenesis, which is crucial for wound healing.

Silk fibroin/PVA blend has also been used; silk fibroin here is the natural component, and PVA being the synthetic component. So, this has been fabricated as nanofibers. Here you can see the silk fibroin-based mat; it helps with cell recruitment, and also, they have done In vivo studies to show improved wound healing and remodeling of the extracellular matrix.

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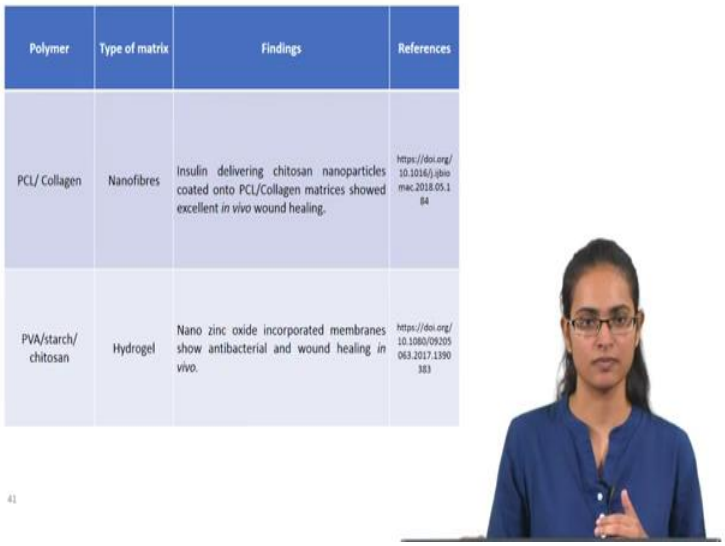

These are other combinations of synthetic and natural polymers. PCL and collagen nanofibers. So, here they have loaded insulin delivering chitosan nanoparticles, which showed excellent in vivo wound healing. And also, more than two components can be blended together to form tissue engineering, skin tissue engineering materials. PVA, starch, chitosan has been used here to form a hydrogel and incorporated with nano zinc oxide to show excellent antibacterial activity and wound healing.

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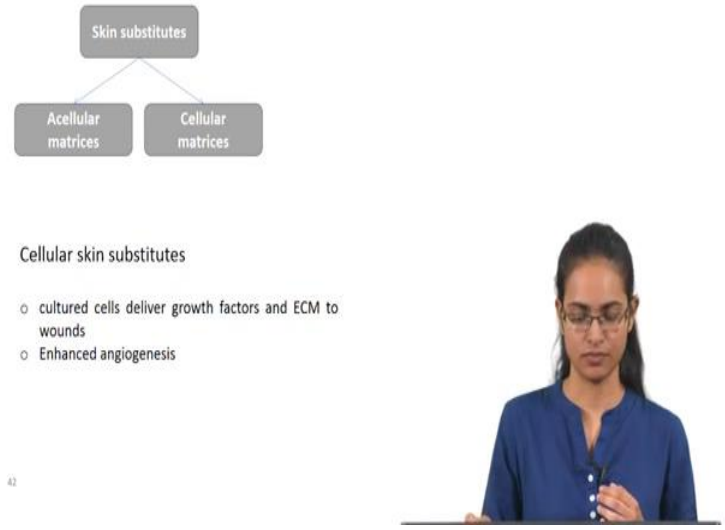

Skin substitutes can be classified as acellular and cellular matrices; the cellular matrices have cells loaded onto the graft. There are certain advantages to that; the cultured cells can deliver growth factors and ECM to the wounds, and also, it has been shown that the cellular skin substitutes can show enhanced angiogenesis.

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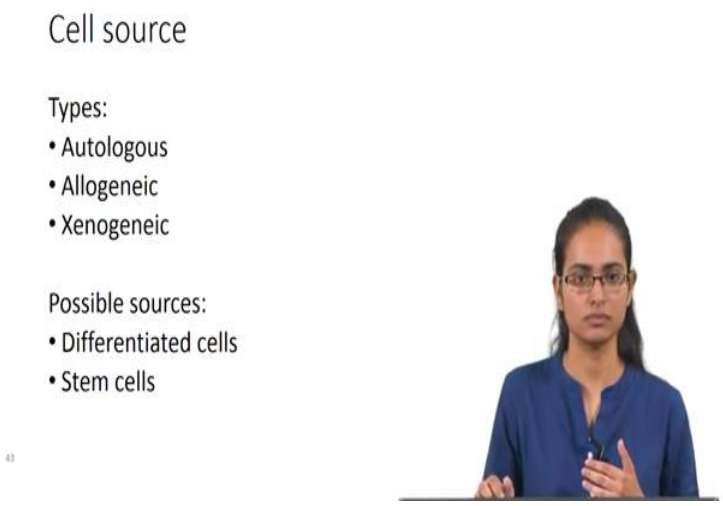

What is the source of the cells that are used for seeding? They can be autologous, allogeneic, or xenogeneic in it, as discussed previously, or they can be differentiated or stem cells.

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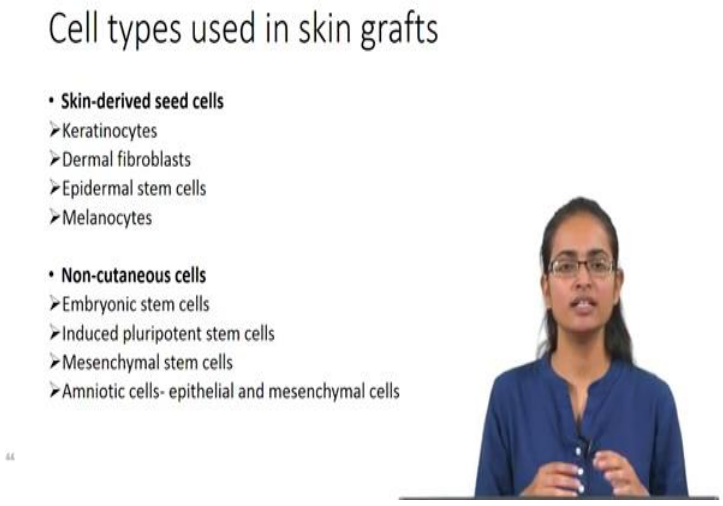

Different cell types are used in skin grafts; they can be skin-derived seed cells, such as keratinocytes, which majorly occupy the epidermal layer. The dermal fibroblasts which lie in the dermis, the epidermal stem cells and the melanocytes which provide pigment to the skin. Non-cutaneous cell such as embryonic stem cells, IPSC that is induced pluripotent stem cells, mesenchymal stem cells, and cells derived from the amnion have also been used.

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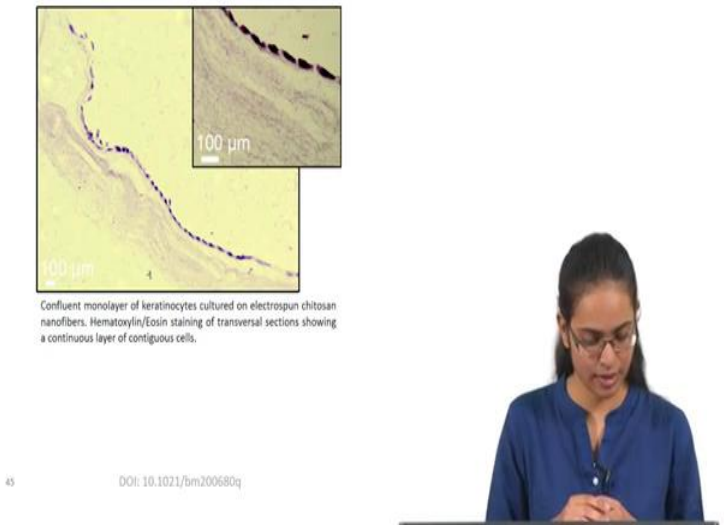

So, this is an image of keratinocytes cultured on electrospun chitosan nanofibers.

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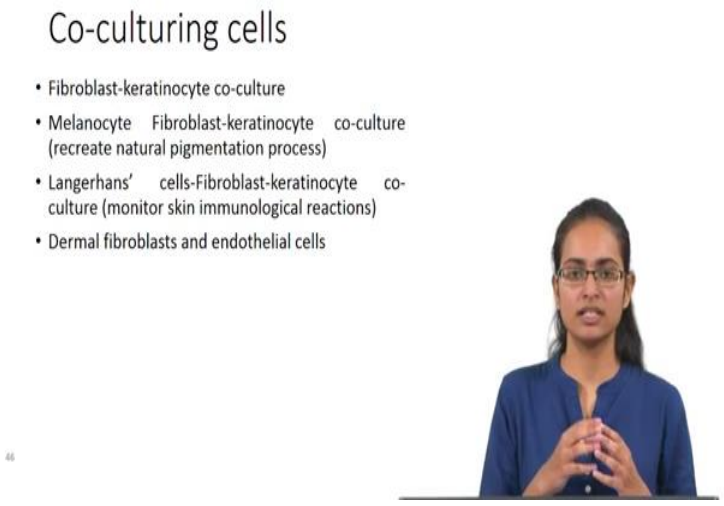

The seeding of cells can be done as a co-culture of two different types of cells. Fibroblasts and keratinocytes, for example, have been co-cultured; and it has been observed that by coculturing them together, the fibroblast can help the proliferation of keratinocytes.

Also, melanocytes have been cocultured with fibroblast and keratinocytes; this can help recreate the natural pigmentation process. Langerhans cells have also been cocultured with fibroblast and keratinocytes. So, this can help monitor skin immunological reactions. Dermal fibroblast has also been cocultured with endothelial cells, so this can promote vascularization.

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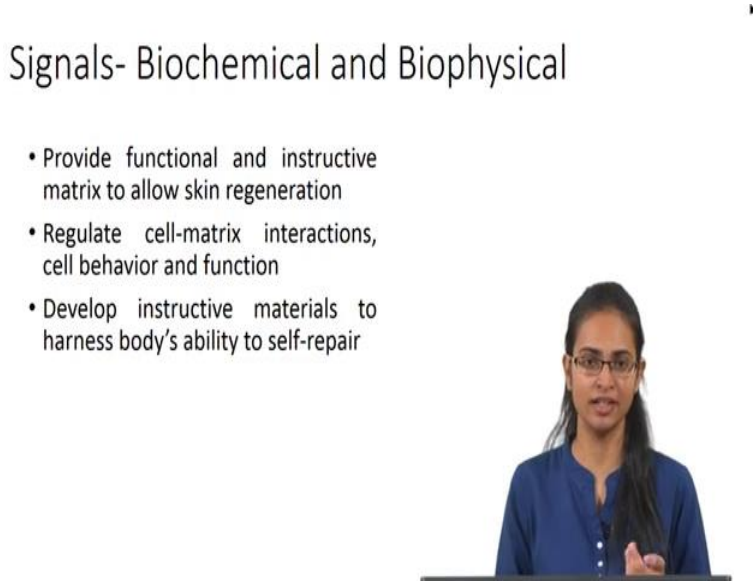

Now, we are going to look at the third component of the tissue engineering triad, which are the signals. These signals can be biochemical or biophysical in nature; these signals provide a functional and instructive matrix to allow skin regeneration. These signals can regulate cell-matrix interactions, cell behavior, and function. So, we can develop instructive materials rather than having passive grafts; these instructive materials can harness the body's innate ability to self-repair.

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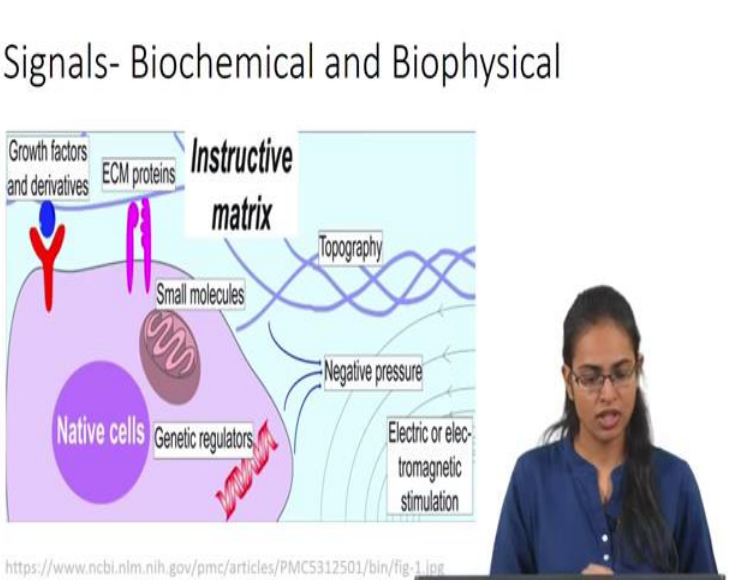

These are the different signals that can be given to the graft. Some of these are biochemical signals, which are growth factors and derivatives, the ECM proteins, small molecules, genetic regulators. And on the right, you can see the biophysical signals such as the topography of the matrix, application of negative pressure, electric or electromagnetic stimulation.

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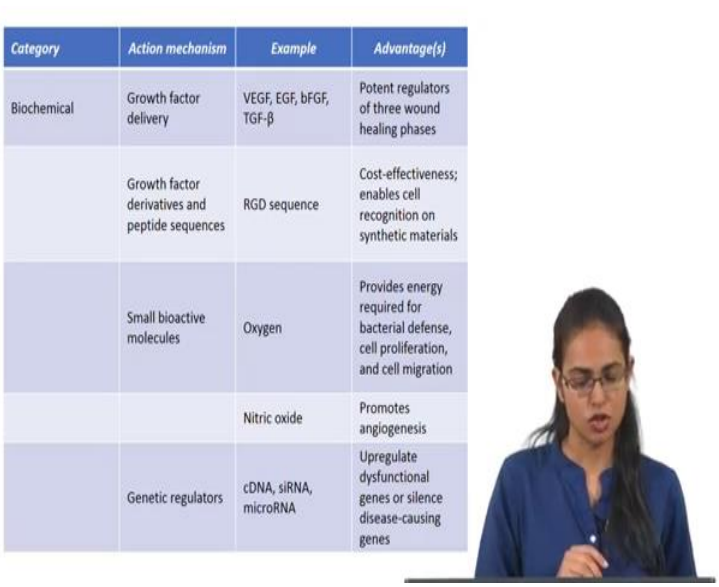

Category	Action mechanism	Example	Advantage(s)
Biochemical	Growth factor delivery	VEGF, EGF, bFGF, TGF-β	Potent regulators of three wound healing phases
	Growth factor derivatives and peptide sequences	RGD sequence	Cost-effectiveness; enables cell recognition on synthetic materials
	Small bioactive molecules	Oxygen	Provides energy required for bacterial defense, cell proliferation, and cell migration
		Nitric oxide	Promotes angiogenesis
	Genetic regulators	cDNA, siRNA, microRNA	Upregulate dysfunctional genes or silence disease-causing genes

First, we will look at biochemical signals. Biochemical signals can be growth factors; growth factors are potent regulators of cellular activities, such as cellular migration and

proliferation and differentiation. Some growth factors that have been looked at for application in wound healing are vascular endothelial growth factor and epidermal growth factor and basic fibroblast growth factor and transforming growth factor-beta. These play a part in a potent role in all three phases of wound healing. Conventionally, these growth factors were delivered using conventional approaches. These conventional delivery methods, however cause burst release of the molecules.

We are developing new techniques to deliver these molecules, which can release the factors in a sustained manner and also so that they can be effective at a lower dosage. One such example can be attaching the growth factors to ECM proteins. Other than growth factors, their derivatives, and certain peptide sequences. The best characterized peptide sequence is the RGD sequence consisting of the amino acids, arginine, glycine, and aspartic acid. So, this sequence is found in several ECM proteins, and also this can be incorporated into synthetic materials, which usually lack cell adhesion sites; this can help in cellular adhesion and migration into the graft.

Other small bioactive molecules can also be incorporated into the graft. One such molecule is oxygen; oxygen levels have been found to be low in chronic wound tissues. Oxygen level is considered important, because it provides energy for bacterial defense, for cell proliferation, and also for cell migration; it is crucial. Oxygen has been used in hyperbaric oxygen therapy, where it is delivered through systemic circulation; however, it fails to reach the desired site. Then they started using oxygen as a topical therapy. Now they have come up with oxygen delivering wound dressings such as oxyzyme, which releases oxygen through a chemical reaction.

Another bioactive molecule that can be incorporated into tissue-engineered grafts is nitric oxide. Nitric oxide is synthesized from nitric oxide synthase, this enzyme is present in 3 isoforms, and all 3 isoforms have been found in the skin; in skin cells such as dermal fibroblasts, keratinocytes. This enzyme is present in 3 isoforms, and two of the isoforms are constitutively expressed and another one is expressed by inducible expression. It is involved in various stages of wound healing, especially angiogenesis; this enzyme is very important for VEGF activity.

Various genetic regulators have also been incorporated into tissue-engineered grafts, such as cDNA, small interfering RNA, and micro RNA; cDNA can be delivered using

various non-viral vectors, such as cationic polymers or liposomes or naked plasmids can be used. cDNA encoding various peptides and growth factors have been incorporated. One such protein is the sonic hedgehog protein; A study showed that the sonic hedgehog gene vector had been shown to improve diabetic wound healing with microvascular remodeling.

Several small interfering RNA has been used. Small interfering RNA can cause silencing of disease-causing genes, by binding to the complementary sequences of the target mRNA. Several diseases such as skin fibrosis, have been treated using small interfering RNA. Another set of gene regulators are micro RNA; these are endogenously found repressors that bind to the three prime and translated regions of the mRNA. These micro RNA have also been used to regulate angiogenesis and also involved in re-epithelialization and tissue remodeling.

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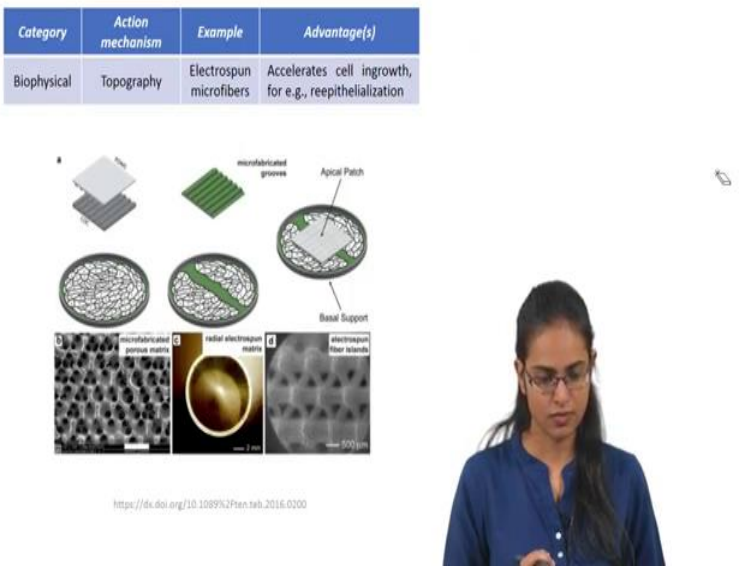

Now, we will look at biophysical signals; one such is the topography of the matrix. The topography of the matrix can guide cell migration through the graft. This can be achieved by using micropatterned surfaces. Here, you can see there is a PDMS membrane, which has microfabricated grooves on it, which has been synthesized using the mold. Human dermal fibroblasts have been cultured on the membrane, and mechanical wounding is done on the surface, and you place your patch on the membrane and observe the cellular in-growth.

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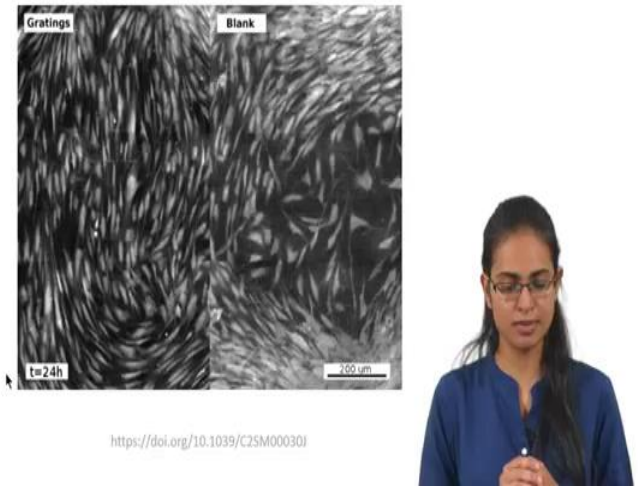

Here, you can see a video. In this video, you can observe the difference in cellular dynamics between grafts that have perpendicular gratings, and other blank grafts that do not. Here at 12 hours, there is considerable in-growth of cells into the graft where the perpendicular gratings are present; while in the blank graft, even after 24 hours of wounding, the graft shows lower confluence compared to the one which has the gratings.

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Another biophysical signal that has been used is the application of negative pressure. This helps in increase in blood flow to the area and also helps in the promotion of

angiogenesis and granulation tissue formation. The mechanism that has been proposed here is, when you apply the negative pressure, there is a slight deformation of the ECM, which generates micro-strain on the cells; this helps in cellular migration and proliferation.

Several vacuum-assisted closure devices have been commercially made available. These devices mainly vary in their filler material that is used. So, either foam or antimicrobial gauze dressing has been used, and they also vary in the suction catheter that has been used. They also vary in the intensity of the negative pressure that has been applied, so which varies from 50 to 125 millimeters of mercury. In this figure, you can see that on the application of negative pressure, there is extruded removal, edema reduction, and blood supply increase that has been shown.

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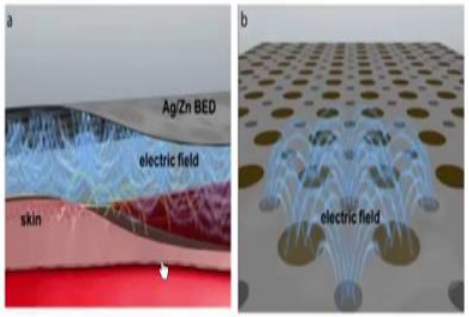
Category	Action mechanism	Advantage(s)
Biophysical	Electric stimulation	Mimics endogenous electric fields that arise after wounding and cause keratinocytes to migrate



Schematic diagram of design, application (a), and electric fields (b) generated by Procellera® bioelectric dressing.

Another biophysical signal used is electric stimulation. This is a biomimicry tool that has been used; it mimics the endogenous electric fields that arise after wounding and cause keratinocyte migration. Procellera is one such bioelectric dressing; it produces electric currents similar to the physiological electric fields present in the body; it produces micro-currents of the range of 2 to 10 microamperes.

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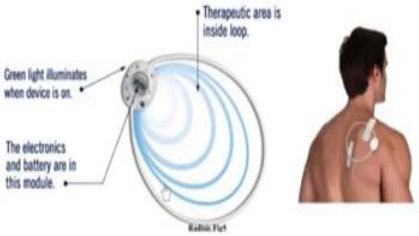
Category	Action mechanism	Advantage(s)
Biophysical	Electromagnetic therapy	Upregulation of multiple growth factors and NO, which regulates cell migration and angiogenesis.



Design and magnetic field generated by RecoveryRx® medical device

Electromagnetic therapy has also been used as the biophysical signal; it resulted in the up-regulation of multiple growth factors, and including nitric oxide, which is very important for angiogenesis. This therapy has also been shown to regulate cellular migration. In the next few lectures, we will look at different fabrication techniques; that have been used to synthesis tissue-engineered skin graft.

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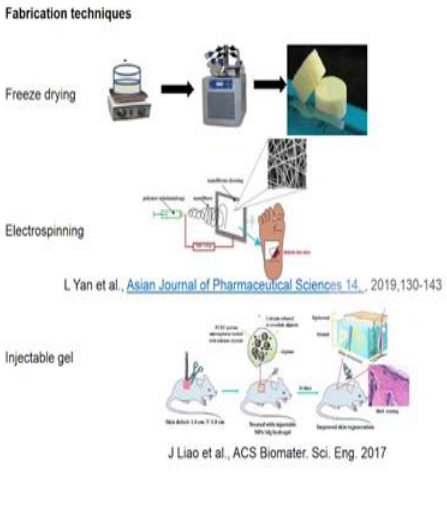

As Vasudha discussed, the design and other characteristics for making skin graft that can be cumulatively made into three categories; the first one is freeze-drying, and the second

one is electrospinning, and the third one is an injectable hydrogel. In freeze-drying, they make the polymer solution, and they will keep it in the frozen condition; based on the melting temperature of that solvent, it will get frozen, and form the crystals. Based on whatever temperature we keep it, that crystal size will be formed, and based on that, the pores will be formed into that scaffold.

In electrospinning, there will be passing that polymer solution under an electric field. When it is spun, and that will be collected into that substrate. So, the fibers whatever is collected will be forming a mesh-like thing, and that can also be used as a skin graft.

In the injectable gel, outside of the physiological environment, that will be in the sol form, when it is injected into that physiological environment that will form the gel. The advantages with the injectable gel over that freeze-dried scaffolds and electrospinning waste scaffolds are; that automatically fills that wound bed, we do not need any specific mold or something, and we do not need any other preprocessing.

So, what we need is, we just need the polymer with or without cells, and that can be directly put into or injected into that place wherever that wound is, and that will fill that wound, and it will form the gel. So, the wound bed will be completely covered.

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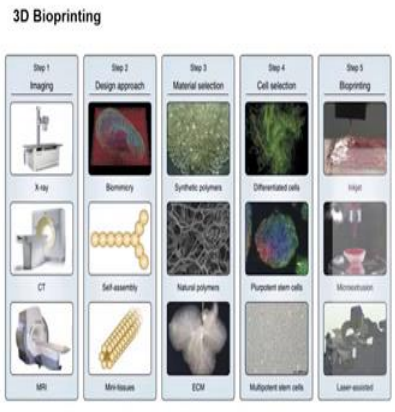

Another advanced technique that is involved with making skin graft is 3D printing. In 3D printing, it involves various steps. First, what we need is, we will be scanning that

particular tissue. If you take skin, that skin will be imaged under a CT scan or MRI scan. So, a CT scan involves an X-ray, and that image will be taken at all the 360-degree angle. In MRI, under the magnetic resonance field, under 360 degrees, the image will be taken. Once that image is taken, that will be converted into a printable form like CAD format. Because then only you will be able to print that particular structure whatever we are interested in.

Then the next step is material selection. In the material selection, whatever criteria Vasudha discussed, we all need to go through all those things, and we need to decide what material we need and for what purpose we need. So, all these things need to be decided.

Then in the case of cell selection, we have epidermis; that epidermis contains keratinocytes, and dermis contain fibroblast. Apart from that, we also can go for some stem cells, as she discussed. Based on our interest, we need to choose keratinocytes or fibroblasts or together keratinocyte and fibroblast; it is up to us like what we are going to make. If you want to make only the epidermal graft, then we need to choose only keratinocytes. If you want to make the dermal graft, we need to choose only the fibroblast; or if you want to make that skin to be in that particular color, we also need to choose melanocytes, and that needs to be co-cultured with the keratinocytes. So, it is all up to us that what we are going to make.

Then the next step is bioprinting. So, bioprinting can be printed three ways; like we can use inject printer or we can go for a micro extrusion-based printer or laser printing. We have already discussed about that 3D printing techniques and how all these things work. So, we do not need to go into detail about those things.

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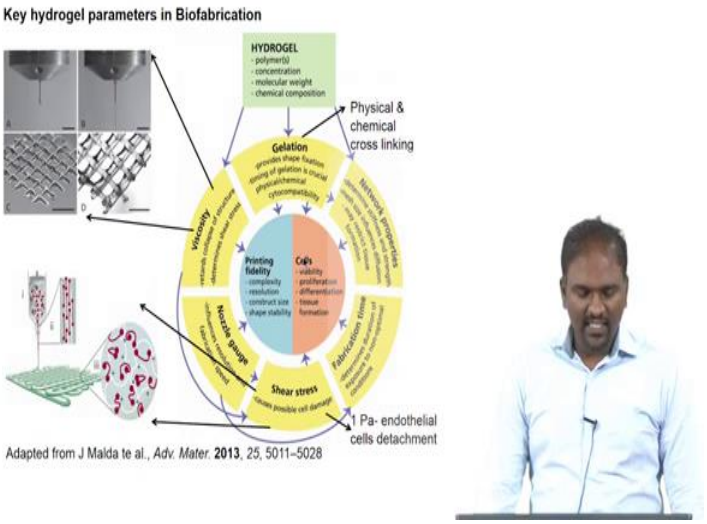

As we know that the hydrogels, whatever we are going to make, that hydrogel should need to be biocompatible. Only that biocompatibility and gelling nature will not help in the biofabrication. So, there are certain criteria that need to be considered. Consider if we form a soft gel, but when you apply some stress or something, the cells will not be viable; then, it is of no use. When we print, it is making a gel; then also it will not be useful. So, the printing fidelity and the speed when the gelation occurs, all these things play a very important role.

All these things are mainly controlled by two main parameters; one is viscosity or the rheology of the polymer whatever we use for biofabrication, and the second thing is the crosslinking mechanism. Viscosity is mainly dependent on two parameters; the first one is the concentration of the polymer, and the second one is the molecular weight of the polymer. These are the two parameters that decide the viscosity of any polymer solution. If we take high viscous polymer with the low molecular weight, what happens? That nozzle will not be able to print that particular thing. If we use that micro extrusion-based technique; we use the needle and that over that needle, we will be applying the pressure. So, when we apply the pressure, that will be pushing that needle, and that will be printing. If that solution is highly viscous, what happens? Instead of making a solution, it will form the droplet. So, to avoid that, what we can go for is, we can mix it with some other polymer, or we can use less concentration of polymer with high molecular weight. So, we can go for this way or that way.

On the left side, we see one fine example. In the example, what they have done was; in the first image A, so they used gelatin methacrylate, which is the known polymer. So, when they used only that gelatin methacrylate, it was not able to print, whereas it formed the droplet; but when they mix that with hyaluronic acid, it formed a clear solution. Once that solution is formed, they were able to get the structure of their interest. So, viscosity plays a very important role in controlling this.

Another thing is that shear stress. In the shear stress, if we apply more shear to the polymer solution; obviously, the cells will get died. There are reports that even if we apply 1 Pascal of shear into that hydrogel containing cells, the endothelial cells get detached from the substrate. Consider even 1 Pascal of pressure is applied, that cells are getting detached; it means, when we apply that shear into that needle or whatever printing technique we use, the cells will not survive. In that, we will get this hydrogel, and we will get the resolution of our interest, but the cells will not survive. In that case, there is no point in doing 3D printing. In the same 1 Pascal of shear stress, they have also found that cartilage function is also lost. To avoid that, what we can do is we can increase the speed of printing and reduce the shear stress.

For example, we have the needle and that needle we are applying some pressure into it. That time of applying that pressure will be less, and the printing speed will be faster. So, the shear whatever is given into that particular polymer solution will become reduced. So, the number of cells that we get will be more, and that will be viable. So, that is the ultimate idea in this.

And the next property with the shear stress is that shear thinning. In the shear-thinning, the polymer solution is in the needle or particular dispenser, so that will be in one structure. When we apply some shear into it that, shear-thinning will happen, and the structure will be reformed or reorganized that will be able to see in the second part of that image; then, once it is printed, that forms particular hydrogel or the structure of our interest. Again, that reformed into that shape whatever we are interested in. If we apply more shear into that particular thing, that reorganization will be a difficult thing. So, we will not be able to achieve that reorganized polymer structure in the substrate. If we are not able to achieve that reorganized structure, the gelation and other things also will get vary. So, these all the main parameters we need to discuss, or we need to know whenever we are going for 3D bioprinting based graft development.

Then the next step is that gelation. As we all know that gelation can be of physical and chemical. In physical, we can use some ionic crosslinkers or that could be of electrostatic based interaction, or we may pass even UV or something, and we get gelation hydrogels. In chemicals, we can also use some crosslinkers, because of that crosslinker that form a gel. So, these are the main parameter we should know before going for bioprinting.

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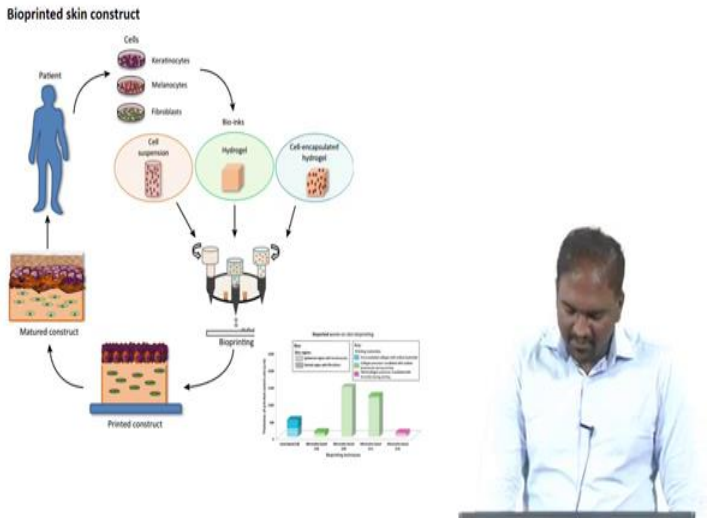

In the case of a 3D bioprinted construct, as we discussed in the design criteria, we need to have the scaffolds. That graft can be of acellular and cellular. If we want to make acellular based scaffold or a graft that we need is only the polymer. That structure whatever we need to bring that will be printed.

If it is of cell-based therapy, then along with the scaffold or hydrogel, we need to culture the keratinocytes and fibroblast or only keratinocyte or only fibroblast according to our interest. They will be grown in vitro, and once they mature, they will be placed into that patient's body, and that will be used as a regenerated skin.

So far, with the various techniques, they were able to achieve that particular thickness. By laser-based technique, they were able to achieve around 600 microns thickness of the skin. In the case of a micro wall-based thing, they achieved about 150 microns. They have used various materials like collagen, hyaluronic acid, chitosan, and various type of biopolymers; especially they are all water-soluble polymers. So far, very limited papers are available with hydrophobic polymers that to be used for skin graft applications.

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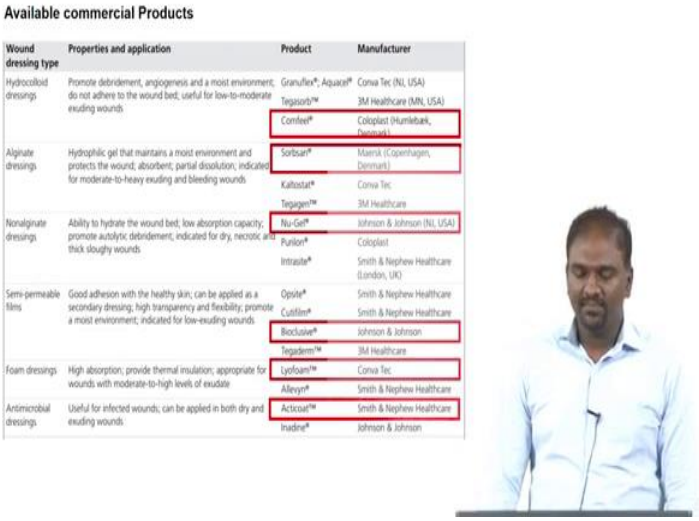

So far, with the design criteria and other things, they have developed some commercial products. Herein we are not just going to discuss about the skin graft based products. Even if there is a wound, they will be needing some dressings. So, even that also we are going to discuss here. The first one is a Hydrocolloid dressing. They will promote that debridement. In the debridement, one of the finest products from that is Comfeel. So, that was developed by Coloplast, Denmark Company. So, what it does is that it will promote debridement, and it will avoid that moisture to go outside.

Then the next second thing is Alginate dressing. That dressing is made of alginate material, and this will act as absorbent. One fine product from that alginate dressing is Sorbsan that was developed by Maersk, Denmark based company.

The next product is non-alginate dressing. In the non-alginate dressings, we have a Nu-Gel developed by Johnson and Johnson. This will again promote debridement, and it also gives that absorption to the wound site. Now that Nu-Gel comes with alginate also just to promote that hydrophilic nature and absorption.

We have Semi-permeable films. Here we have Bioclusive developed by Johnson and Johnson. Herein we have a polyurethane film. So, that polyurethane film is coated with acrylic adhesive. When we place into the wound, that will automatically adhere to the wound site, and it will pass that air to pass, and that will keep maintaining that moisture

condition. If it is dried, it will be difficult for that wound to heal. So, to maintain that, they use semi-permeable films.

They have foam dressings also, as we all know, foams are mainly made of polyurethane. This is just to protect from the thermal environment; from the various temperature, it can be protected. So, that is made of polyurethane.

We have antimicrobial dressings. As that name indicates that dressing is to avoid that infection, whatever microorganism invading into that body that can be avoided by these types of dressings. Acticoat is developed by Smith and Nephew Healthcare, and that is one of the products which is available. So, these are all the products whichever is available for simply wound dressing, not for the graft.

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Substitute	Product	Graft type	Cell source	Manufacturer
Epidermal substitutes	Epicel®	Cell based	Autologous keratinocytes	Genzyme Biosurgery (MA, USA)
	CellSpray®	Cell based	Autologous keratinocytes	Avita Medical (Perth, Australia)
	Myskin™	Scaffold containing cells	Autologous keratinocytes	CellTran Ltd (Sheffield, UK)
	Laserskin®	Scaffold containing cells	Autologous keratinocytes	Fidia Advanced Biopolymers (Abano Terme, Italy)
	ReCell®	Autologous epidermal cell suspension	Autologous keratinocytes	Avita Medical
Dermal substitutes	Integra®	Cell free	–	Integra NeuroSciences (NJ, USA)
	AlloDerm®	Cell free	–	LifeCell Corp. (NJ, USA)
	Hyalomatrix PA®	Cell free	–	Fidia Advanced Biopolymers
	Dermagraft®	Scaffold containing cells	Neonatal allogeneic fibroblasts	Advanced BioHealing (CT, USA)
	TransCyte®	Scaffold containing cells	Neonatal allogeneic fibroblasts	Advanced BioHealing
	Hyalograft 3D™	Scaffold containing cells	Autologous fibroblasts	Fidia Advanced Biopolymers
Dermoepidermal substitutes	OrCel®	Natural-based scaffold containing cells	Allogeneic keratinocytes and fibroblasts	Ortec International (GA, USA)
	Apligraf®	Natural-based scaffold containing cells	Allogeneic keratinocytes and fibroblasts	Organogenesis Inc. (MA, USA)
	PolyActive®	Synthetic scaffold containing cells	Autologous keratinocytes and fibroblasts	HC Implants BV (Leiden, The Netherlands)

Adapted from Periera et al, *Nanomedicine* (2013) **8**(4), 603–621

When we come to the graft, the graft can be of split-thickness graft or full-thickness graft. Split-thickness graft, even that could be of dermal graft or epidermal graft. The full-thickness graft will have epidermis as well as the dermis. The grafts can be of acellular as well as cellular. So, all these things people have developed and they have the products.

Epidermal grafts they are mostly of cell-based grafts. So, few people use some scaffolds; whereas, few do not. Epicel and Cell spray are simply cell-based things; what they do is,

they only supply the cells into the wound, and they will start growing. The Myskin and Laserskin they have the scaffolds. So, here they use collagen and alginate-based thing.

Dermal substitute, so herein one of the successful products which is Integra developed by that John group. We will be discussing about how that works. Apart from that, Alloderm, Hyalomatrix made of hyaluronic acid, Dermagraft, TransCyte, and Hyalograft all these products are commercially available. In the case of a full-thickness graft, we have OrCel, Apligraf, and PolyActive. So, the companies whichever is developed also it is given.

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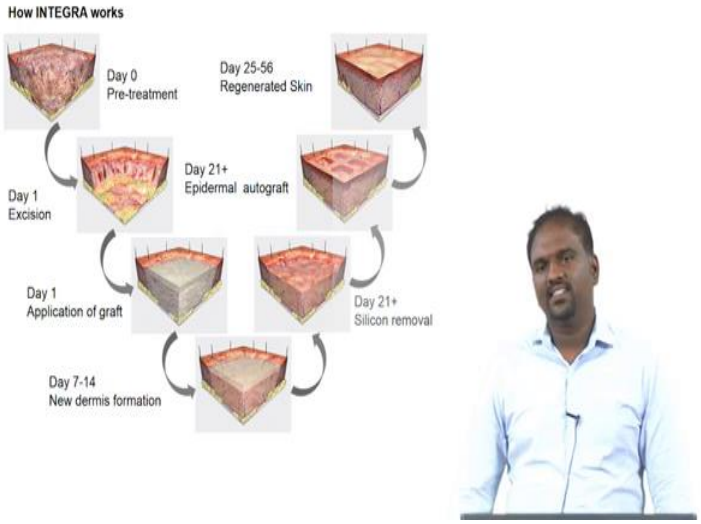

As I told, Integra is one of the finest and successful products in the market. It develops only that dermis part. It involves dermis development by the scaffold and epidermis development by the autologous skin. At the first day, consider this is the wound. What they do is, they clean that debris whatever is present at the wound site. Then, they will cut that portion wherever that wound extended. Once everything is done, they will be placing that graft. So, that graft contains that collagen and hyaluronic acid as a bed, and over there, we have the silicon layer.

After the 7th day, new dermis will be formed. Once that new dermis is formed, they will remove that silicon layer, whatever is present on the top. The silicon layer is given just to avoid the contraction and to avoid that moisture to go outside of the wound bed. Once that silicon is removed, they will take that autograft by dermatome or any other

technique, they will just cut that tissue from the wounded patient, and they will place it over that dermis. From 25 to 56 days, we get the regenerated skin; this is how this Integra works.

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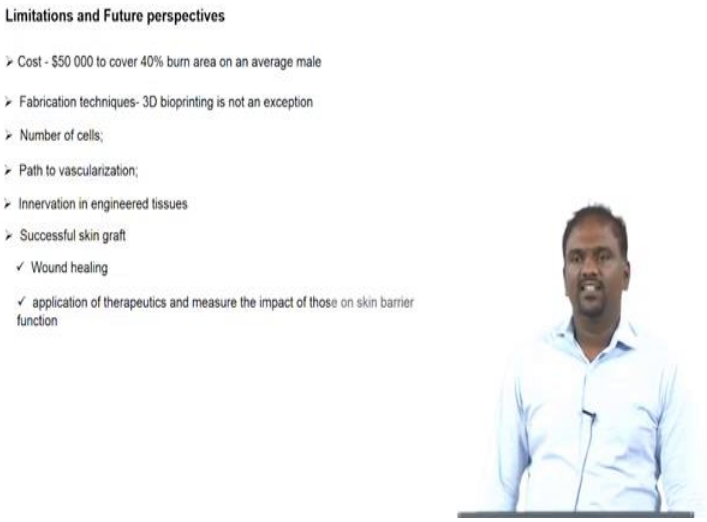

So far, we discussed about that skin graft, design, and why it is required and what are the techniques involved in developing such skin graft. So far, there are so many studies that have been done, and they have tackled so much of the problem. Still, there are some problems that exist. One of the main problems is the cost. If we consider that cost in the US dollar. 50000 US dollars is needed, just to cover that 40 percent of the wound for a male patient. So, this is costlier. To avoid that, we need to go for some easy strategy to make some skin graft.

Then obviously, those fabrication techniques are also a very limiting factor, so even 3D printing was not an exception. In 3D printing, resolution and other things play a role. So, this needs to be optimized, so that we will be getting the proper skin graft. Then the next is the number of cells. As she discussed, the choosing of cells and the number of cells whatever we are going to encapsulate into the hydrogel also matters; because if we seed a very less number of cells, that will not proliferate, and that will not do the function of our interest.

Also, we should not take a higher number. Even if we take higher the number then unnecessary fibrillation and other things will happen, because of that, the wound will get

spoil. An appropriate number of cells need to be chosen, and that also another limiting factor.

As I told that in the anatomy section, vascularization plays an important role; because of that vascularization only the cells are growing into the top direction, say in the epidermis from epithelial cells to keratinocytes and further the next to next layer and finally, the cells are getting died, and they form the shed of the cells. If that proper vascularization is not there, the signals or nutrients will not pass into one layer to another layer.

Achieving vascularization can also be done by applying negative pressure and some electric field, as we discussed in the previous session. Still, it plays a very limiting role in the vascularization part. Innervation is another important factor we need to consider, not just for skin with all the grafts we have this problem. So, this also another limiting factor for this skin.

Once that successful skin graft is developed, we can use it for wound healing on accident or burns wound, or a diabetic wound. Also, it can be used for screening various drugs that could be used for various cosmetic applications.

Thank you.