3D Cell Cultures & Synthetic Skin

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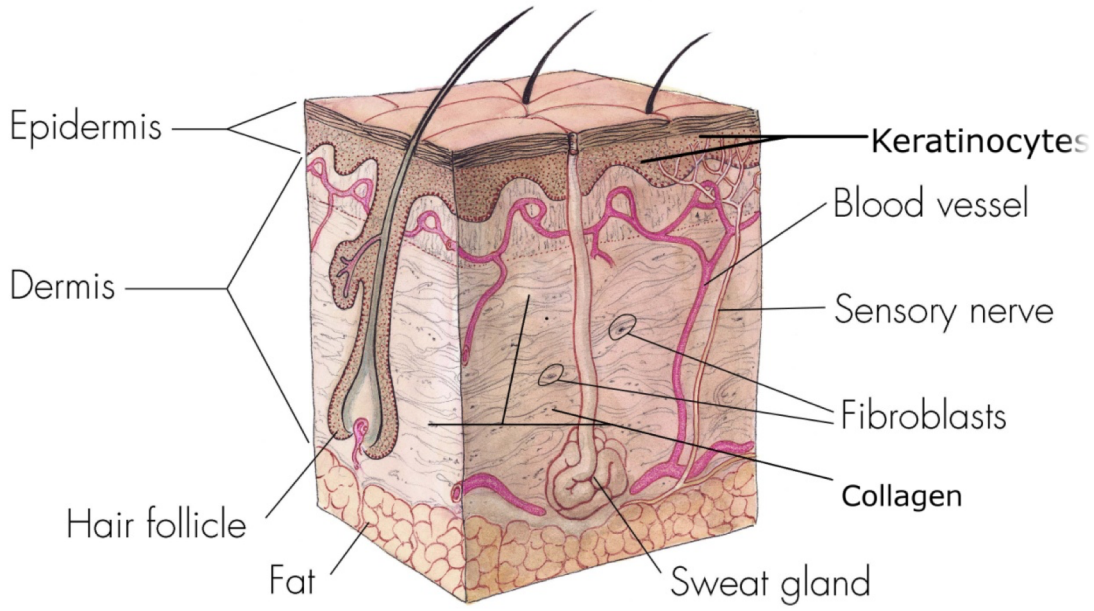
Preface

We wanted to handle a topic where we would learn something completely new and where the topic was relatively unknown to the internet. 3D cell cultures will one day be the norm for drug testing and will enable us to no longer have to do animal testing.
We had no idea about 3D cell cultures before starting our project. Learning about 3D cell cultures was extremely interesting. We didn't even know that growing tissue in a Petri dish didn't lead to 3D cell structures, like in reality.
What is 3D cell culture and how can you make synthetic skin out of it? What is needed to produce synthetic skin? Why are people so interested in 3D cell cultures?

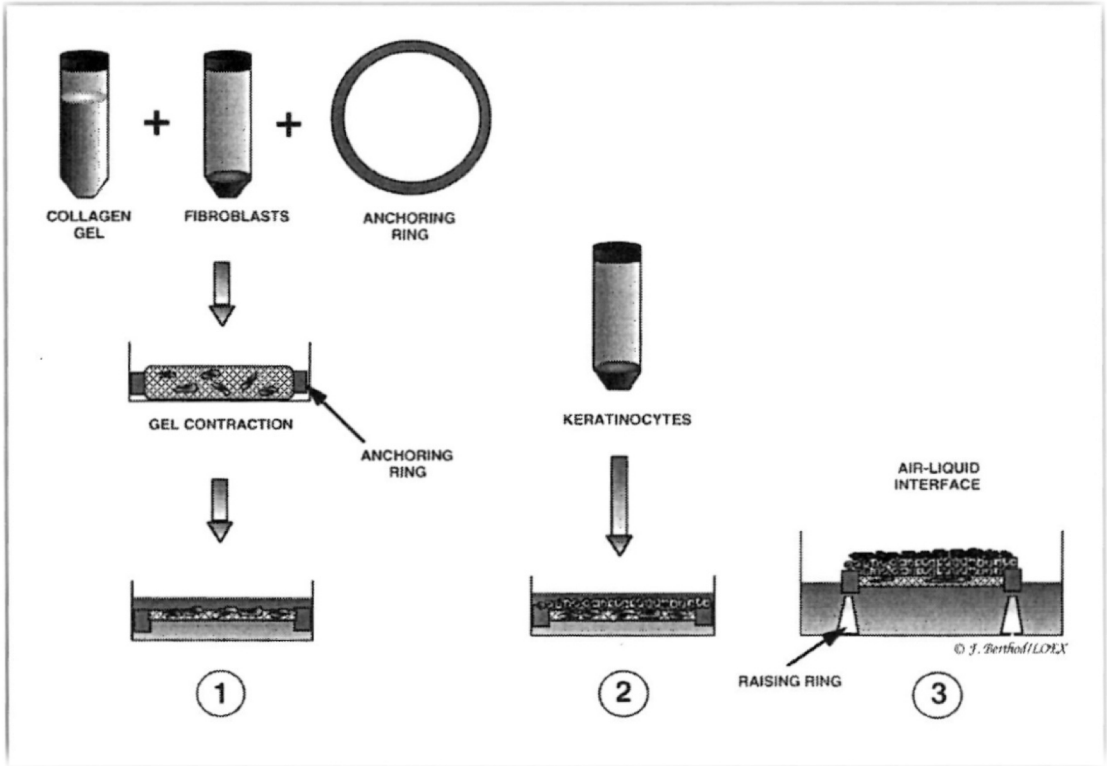
Introduction

Our skin creates a barrier between the inside of our body and the outside world. It protects us from disease and foreign materials, but it also has to be very sensitive, so that we can feel and differentiate between hot and cold.
Our epidermis renews itself every 26-28 days. Everyone is aware of the skins' great healing ability, having noticed a small cut heal in days. Scars might remain, if the cut was deep enough (only the epidermis heals itself). But if the injuries are too extensive (a surface of about 4 cm), then the skin can't heal itself completely.
Any new cosmetics (drugs in general) must be tested for. This is mostly done on animals, a long debated issue, which is of great importance. A more ethical and practical solution, is offered by the production of artificial skin. For the past 30 years, tissue engineering has been the order of the day and has made a lot of advances. Previously, skin from flaps or the scalp were surgically removed and grafted on to a damaged area. However, we want to focus on more relevant and new technologies. This includes the development of 3D cell cultures. The breakthrough was made when the dermis and the epidermis were first synthesized, since the cells accumulate at the dermis, thereby forming the epidermis. There is an important difference between the cultivation of cells on a two-dimensional substrate or in a three-dimensional environment. This allows you to recreate the various layers of the skin and to create its structure. This was achieved with a gel, specifically a gel made out of collagen. This enabled the testing of drugs in an ex vivo environment.
A new project is under way in the Children's Hospital of Zurich, where they want to implant fully functional skin on to 20 children, who were victims of a fire.

Engineering Technique

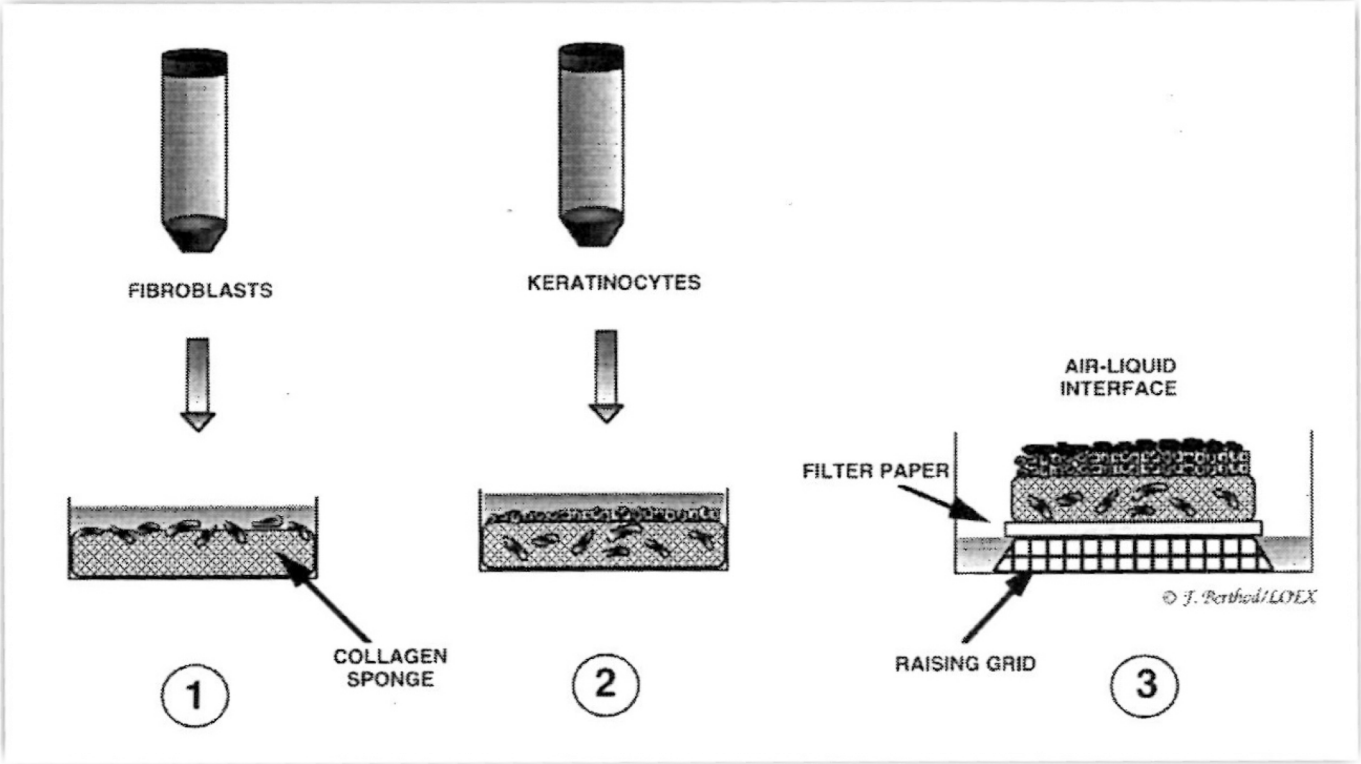


A cross section of real human skin so that similarities and differences can be seen. They are very many ways to grow synthetic skin in a 3D cell culture. We will describe the two simplest forms.
The first is a skin substitute made from a collagen gel.



The major protein found in the human dermis is collagen and since it is easily extracted from rat tail tendons it has been readily used as a matrix for 3D skin cell

cultures. Fibroblasts are embedded in the collagen gel at the appropriate physicochemical conditions which then recreates the dermal layer. The Collagen Fibroblast mixture is poured in a paper filter ring, in a Petri dish, to which the mixture attaches. This allows the preservation of the surface area, even though the gel contracts due to the fibroblasts, with only thickness decreasing (1). Keratinocytes are seeded on top to create the epidermal layer (2). Finally, the substitute is rising to the air-liquid interface to promote epidermal differentiation (3).



The second is a skin substitute made in a collagen Sponge.

The Sponge is made by mixing different collagen types and other substances, the solution is then poured in to plates, frozen at -70°C and lyophilized. This collagen sponge provides a porous structure into which the seeded fibroblasts can creep (1). Keratinocytes can then be deposited on the upper planar surface made by the fibroblasts (2). The advantage of this model is the production of a high amount of newly synthesized extracellular matrix that fills the sponge's spores after one month.

Interview

Our Interview was with Heinz Ruffner Ph.D Labhead NIBR Novartis Pharma AG. On the Novartis Campus

Q: To begin with, your Work is on 3D cell structures. As I understand, it involves a gel which acts like scaffolding for the cells. Is this correct?

A: Yes that is correct, the scaffold basically is used to keep the cells in place, to give them an anchor. Cell support is needed so that the cells, when they bind to the scaffold can initiate transduction of some signals from the outside of the cell to the inside of the cell. So in that case the scaffold also provides means to the cells for signalling.

Q: This gel, this "scaffold" how does it affect the result for when testing the culture with drugs?

A: It certainly has an effect on the culture, because if you would take a material which is completely inert, so something that doesn't have a functional group, then the culture system would not work. So what you need is a 3D culture which contains some proteins which have some certain biological activity, e.g. a collagen, collagen 1 in particular is a protein which is needed by the cells for signalling, if you would take another 3D matrix it would not work. It depends, for some of the cultures you also need to add some factors, some growth factors e.g. that are needed in addition to what's on the matrix supply.

Q: How similar are your 3D cell cultures to the real thing?

A: The cultures are always an approximation of what happens in vivo, this is clear, and what you can do with such structures is to mimic some of the processes which naturally occur, but you cannot mimic all of them. The easiest for a skin cell culture is start to grow Fibroblast cell layer in a certain matrix like a collagen matrix and then you overlay a layer of Keratinocytes on those and then you let the Keratinocytes stratify under certain conditions, so that you basically create an epidermis made out of Keratinocytes and a dermal compartment, a dermis made out of collagen and of Fibroblast and so that's the easiest. It's good enough that if you want to study e.g. the affect some compounds have on Keratinocytes or on Fibroblast or on their interaction. However such structures will never make sweat glands, they will never make blood vessels or hairs etc. So if you want to study that then they're not sufficient. So it really depends on what you want, to study Keratinocytes or dermal interactions then some of those systems are sufficient but if you want to study other processes then they're not. They are however ways where you can introduce for example Endothelial cells which are blood vessel cells so that you can the study those processes as well, but in the end its certainly not living tissue so they are differences.

Q: Stem cells play a big part in 3D cell cultures, would you say that the stem cells are only needed in the current stage of 3D cell culture technology and later on it won't be a requirement, or is it more of a permanent part of 3D cell cultures?

A: We use the stem cells to create the in vitro or ex vivo cell culture systems and I think they will be always needed because if for example you want a skin culture you need a certain amount of stem cells or progenitor cells, so that they repopulate the

cell pool which you have, so that you get more cells and on the other hand you need them as a precursor for the differentiated cells, so in terms of Keratinocytes you will always need stem cells to seed in order to make 3D cultures, but there are stem cells present in our skin throughout our life, so when you make cultures like that you will always need stem cells.

Q: How good can your cell cultures become in regard to drug testing, will it ever become as good as in vivo or will you always need a combination of both?

A: Well in my personal opinion I think you will be able to go very far with these cultures and not have to use in vivo (animal experiments) anymore. However the current system (of 3D cell cultures) that people use isn't sufficient because one serious issue is the barrier function of the epidermis is not present yet really on a 3D skin equivalent or a 3D cell culture but I think people are really working on this quite hard in order to increase the barrier of the skin. I mean if you would apply a cream on a 3D cell culture the drug would just go in the skin and infuse and that would not be a really meaningful in term of assessing or testing your drug. So I think what you need to do is to improve that and there are methods to do that and people also have made progress already. I see that those cultures systems will eventually replace fully what people have been doing before with animal experimentation.

Q: Right now what barriers, limitations face 3D cell cultures?

A: Some of the currently available cultures do not allow for hair growth which is a important part of the skin because the hair follicle contains stem cells which are need not only for the hair and also for the inter follicle epidermis, for other parts of the skin and you don't have sweat glands, melanocytes are not there. I think the limitation is such that the complexity of the system is not comparable to in vivo. So you can only study affects on the cell that you put in your in-vivo system but you don't know how the other cell types which you don't have in your in-vitro system would behave.

Q: These are however not permanent limitations?

A: I think so, like I said hair follicle generation is something one could address, melanocytes have been brought into cultures, endothelial cells have been reported that some groups have brought in. In the end I think it will depend on how functional it is, but in terms of complexity I don't think that it will be a big issue anymore in the future. The other limitation may be scalability, can you really scale a 3D culture in such a way that you could do a large screen, I think that would be another limitation, but there progress also has been made.

Q: Will these 3D cell cultures help in growing fully proportioned organs? Or is this not the aim of 3D cell cultures?

A: That's an interesting question, I think both applications are in the minds of people. There was just a recent report by the University of Zurich where they have been able to grow 3D cell cultures made out of fibroblast collagen and Keratinocytes which they will transplant onto patients, children with large skin defects due to burns. So that is one aim but the other aim is certainly to use such systems as a drug discovery tool and I think both are important.

Q: Do you specifically work on creating these 3D cell cultures or do you also do drug testing, with them?

A: No we are working on establishing the cultures so that we can use them to screen for compounds for drugs. The goal is to establish a system which closely mimics the skin of a human being so that you really can have drug screening done on such 3D cultures so that the chance that your drug will work later on, on patients is as high as possible. So in other words the better the system you establish, which we are hoping to do, the higher the chance is afterwards that whatever you identify as small molecules as compounds will work in the patient. That's very important here because, I think traditionally in drug discovery people have screened for small molecules in very simple systems and then the chance that afterwards that such a molecule will work in a patient was very, very small, because they are so many steps in between. Our aim is that by screening in a system which very closely mimics human skin that whatever you will identify in such a system as a positive compound will have a high chance to also work in a patient.

Q: Right if you would test a drug only on your 3D cell cultures could it get approved or do you also have to test in vivo.

A: I guess it would also have to get tested in terms of toxicology, for example they are several test which you have to do, so the FDA will require that you have toxicology tests done, there will be battery of tests which you have to do until it gets approved including testing in healthy volunteers etc.

Q: 3D cell cultures still have their validity or are they still in the development stage?

A: Our culture systems are relatively new and I think people have to get used to them first and see what value they bring. Certainly you will see that the compounds which you will score positive in your assay will be beneficial later on in clinical trials. The value of the 3D culture system will increase as well, so it's still at an early stage, but in the end whether you have screened in a 3D cell culture or in a conventional system it doesn't really matter. What counts is that in the clinical phases that you have an efficiency and efficacy of the drug compounds. Our goal really is just to get faster to the clinical trials and also to have less compounds which will fail in the clinical trials.

Q: So you said that 3D cell culture technology is quite new, what exactly is new.

A: You're certainly right 3D cell cultures have been around for quite a bit maybe 20 years or so. I think what people have been doing 20 years ago, is to take epithelial cell from the breast tissue, female breast tissue and they have grown cells in spheres. So that was something where you had a 3D culture system, but it was kind of an unnatural culture system which was generated, but still it had some advantages. And the people have tried to make skin cultures those attempts have been also relatively old maybe 15-20 years, where people have grown Fibroblasts in Collagen and then overlaid with Keratinocytes that was really only done for basic research. I think what changed now is that those structures will really be used for drug discovery. Its scalability which you are doing now on such culture systems, its advancements in how you grow those culture systems, it's the complexity on which the people are working right now and what is new it's that people realize now that you can really use them for drug discovery, whereas before it was for basic research.

Q: Is there a drug that you're testing on your 3Dcell cultures to basically showcase 3D cell culture technology, in that it will or has scored high in clinical trials?

A: Absolutely I think that's what you need to do, it depends on what you want to do afterwards in patient and disease treatment. But there is not a lot on the market actually which works and has shown efficacy, certainly not in terms of small molecules and they are few proteins which people have been using, growth factors mainly in the past which has shown some efficacy, but there is not a lot, but there are some which have shown weak efficacy like PDGF, pigment derived epithelial growth factors, which was approved for some treatments that is one biological compound that one should test in 3D system. I think that's a benchmark, you need what has been shown to work already, needs to be shown working in a 3D cell system.

We weren't allowed to take any pictures of the Labs (a Novartis policy) we did however receive some stock photos of the Labs.

Discussion

With new advancements in 3D cell culture technology, synthetic skin can be recreated to a degree where it is very similar to the real thing. This enabled due to the three-dimensional space, which allows the cells to form in layers and complex structures.
Of course the 3D cell culture grown skin isn't like real skin. There are many details, which the 3D cell culture grown skin doesn't have. Very important for the skin are the hairs within the skin. The hairs protect the skin against light (UV- & Infrared-light) and they have an influence on the temperature regulation of the body.
Also the artificial skin isn't able to sweat, it doesn't contain any sweat glands.
So the scientists have to find methods to synthesize hair cells and sweat glands, which will enable the 3D cell culture grown skin to be more like real skin.

Advantages:

- Drugs can be tested on synthetic skin, nearly no animal testing would be necessary which would save many animal's lives.
- In addition, this way drugs will be approved faster and patients will receive appropriate treatment earlier.
- The produced synthetic skin can be used for patients, who have serious burns, cauterization injuries or skin diseases like skin cancer.
- Collagen can be produced easily.

Disadvantages:

- There is also a risk, that the patients' immune system rejects the synthetic skin.
- Stem cells are needed, they are taken from aborted fetuses, which is an ethical problem in certain circumstances.
- You can't include sweat glands, hair cells and blood vessels in the synthetic skin.
- The complexity of the system is limited.

Summary

Skin growth in a 3D cell culture can be grown in two different ways. Either with a collagen gel, where gel and Fibroblasts are mixed and then applied to a Petri dish and then keratinocytes are applied on top. The other is with a collagen sponge where Fibroblasts are seeded in and the keratinocytes are applied on top. The major advantage of this technique is that the Fibroblasts produce new extracellular matrix that fill the sponge's spores.

These 3D cell cultures of skin resemble real skin in that these layers of cells are present and some of the interactions between them are to. But sweat glands, hair follicles and blood vessels are not present. This means that the complexity of the cultures is not unlimited. However advancements are being made which means that these limitations are not permanent.

With all of the advancements made in 3D cell cultures, they are starting to become more viable as a method for drug testing. This is what Heinz Ruffner and his team wants to do. Their current goal is to improve a drugs clinical trial testing by doing extensive drug screening, to see whether a drug is viable or not. Of course this means advancements in 3D cell culture technology and the acceleration in the development of the right kind of drugs.

3D cell cultures have like everything else their advantages and disadvantages, however most of the disadvantages can be overcome with time, except for one. Using stem cells seems to be unavoidable, it being required for all the differentiated cells. But this dilemma is of ethical kind which for some isn't a major stumbling block.

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Glossary

- Transduction: Transfer of genetic material
- Matrix: The tissue between cells
- Fibroblast: Cells, which produce collagen and other proteins
- Keratinocytes: Skin cells, which produce keratin
- Stem cells: An unspecialized cell, which are able to acquire a special function by cell differentiation
- Progenitor cells: Are like stem cells, but it's more specialized than stem cells
- Melanocytes: Cells in the epidermis, which produce melanin
- In Vivo: As in real life
- Ex Vivo & In vitro: In an artificial environment outside the living being