24/04/2020

AURICULAR CARTILAGE RECONSTRUCTION

Using Apples as a Scaffold

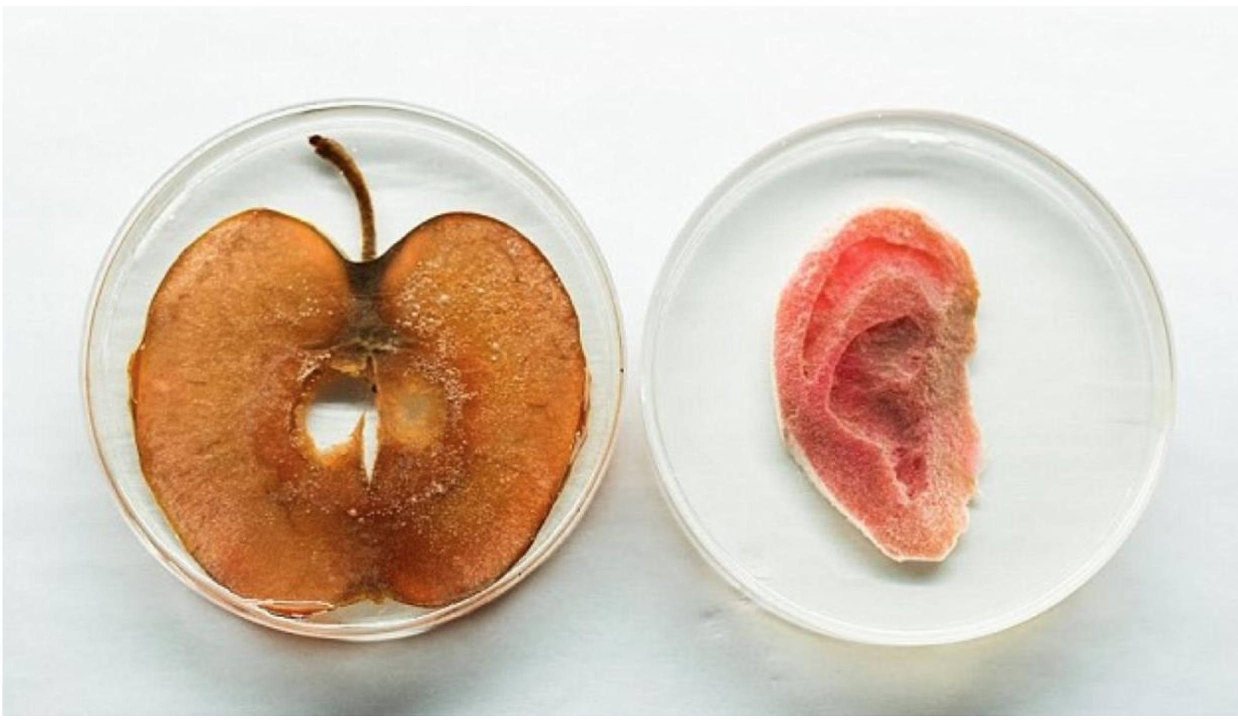

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Table of Contents

1. Preface

Biotechnology is a very complex but interesting topic to study. As we both find medicine a fascinating subject, we started brainstorming and searched for issues in modern-day medicine and existing solutions, or rather approaches, with the help of biotechnology. After doing our research for a while, we stumbled across an article which covers the following topic: creating an auricular scaffold made of apples with the application of regenerative medicine. Our curiosity intensified after we found out that fruits and vegetables can be used for this scientific procedure.

The following paper will explain and discuss the creation and futuristic outlook of an auricular cartilage with the help of apples by answering the following questions:

1. What is auricular cartilage tissue engineering?
2. What is “the apple phenomenon”?
3. Is this invention revolutionary?

Moreover, a glossary which can be found in the appendix serves as a guide, which defines specific terms. The information for this paper will not only be taken from books and internet articles, but also from an interview we held with I.M., a specialist for tissue engineering. Our motivation behind our work lies with the fact that even modern-day medicine is facing issues which are still hard to solve including the deficiency of perhaps organ donors or having trouble to replace dead body parts of a human being. We find that if it were truly possible to regenerate, respectively replace, body parts with normal fruits and vegetables, a huge success for the healthcare industry is about to arrive.

2. Introduction

Hearing is essential in helping us socialize and communicate with people and therefore also helps us function properly in our every-day lives. Furthermore, it can save our lives when life-threatening alerts are brought over in an auditive way such as an alarm. But what if you no longer have the ability to hear as well as you used to? What if your ears were injured in an accident? This is when regenerative medicine comes to help. In this paper, a unique approach to this problem will be explained:

The functioning and value of the auricle is not made clear, i.e., directional hearing and aesthetics.

Figure 1: Andrew Pelling at TED 2016

Scientist, entrepreneur, professor and biohacker Andrew Pelling perhaps found a way to solve this problem. The history behind everything, is that Pelling likes to look through people’s garbage. For him, garbage, mainly hardware, is a chance to play and create things. Because it is what he likes doing, he made it a part of his day job. He leads a university based biological research lab where the people there are not trying to solve any particular problem but just want to be creative (TED 2016). As Pelling himself said: *“What happened, was that artists and scientists started coming to my lab. And it’s not because we value unconventional ideas, it’s because we test and validate them with scientific rigor.”* (Pelling 2016). So, one day, Pelling was hacking something and then he asked himself if he could treat biology like

This reference does not exist.

hardware. If he could just mix and match parts in a specific way and create something with it. The result was something worth talking about.

2.1 Regenerative Medicine and Alternatives

Before diving deeper into this topic, one should understand the differences between regenerative medicine and tissue engineering.

Regenerative medicine is a medical field, which aims to restore, replace or regenerate tissue or organs which have been damaged due to injuries, diseases or natural aging processes and therefore no longer have the capability of fulfilling their functions for the body. So, auricular cartilage reconstruction falls into this medical field. Oftentimes, most life-threatening diseases and illnesses such as cancer, are currently treated with palliative therapies. While these treatments can be seen as an alternative to regenerative medicine, they only decelerate the patient's diseases or illnesses and are very costly. Regenerative medicine is one of perhaps many solutions to a few health conditions, although there are many factors which play a role in making this medical procedure succeed (Alliance for Regenerative Medicine (year unknown, p.3).

„only“, well they improve the patient's life.

2.2 Tissue Engineering

Whilst regenerative medicine is practiced in vivo, in other words within a living organism such as an apple, tissue engineering aims to repair tissue in vitro. This scientific field focuses on combining scaffolds with cells and biologically active molecules into functional tissues. It aims to assemble these constructs in order to augment, maintain or improve damaged tissues, such as an auricular cartilage, or even whole organs (National Institute of Biomedical Imaging and Bioengineering, year unknown).

2.2.1 What is auricular cartilage tissue engineering?

Auricular cartilage tissue engineering is the science and creation of the cartilage of an auricle. The goal is to reconstruct a natural looking ear with the same characteristics as a cartilage (Murthy 2019, p. 291-302). The detailed procedure and the problems scientists are facing even today, will be explained in the following sections.

3. Engineering Technique

3.1 What is the apple phenomenon?

Basically, Andrew Pelling is trying to prove that organs like the auricle can be reproduced by using fruits such as apples as a scaffold. He contended that commercial scaffolds are very expensive and problematic because they derive from proprietary products, animals or cadavers which are more expensive than fruits. “*We took a totally innocent Macintosh Apple, removed all the apple cells and DNA and implanted human cells. What we're left with after removing all the apple cells, is the cellulose scaffold”* (Pelling 2016). Pelling explains that the key to remove apple

Figure 2: According to Andrew Pelling, the cells turned red after seeding mammalian cells.

cells and DNA, is only soap and water. Pelling added that human cells should be implanted which start to multiply on the scaffold (see figure 2). With this cellulose scaffold made from an apple, he wonders, if it were possible to create functioning body parts out of things from our everyday lives we can find in our kitchens (Pelling 2016).

3.2 Structure and Properties of Cartilages

First of all, it has to be mentioned that the external ear consists of elastic cartilage which is covered by a skin called perichondrium. "*Cartilage is made up of specialized cells called chondrocytes. These chondrocytes produce large amounts of extracellular matrix composed of collagen fibres" (*Mandal 2019, www.new-medical.net). Unfortunately, the adult human cartilage shows a poor capacity for self-repair and regeneration because of highly differentiated, non-dividing chondrocytes (Pintanguy et al. 2009, p. 447). This and the three-dimensional complexity are what makes auricular cartilage reconstruction one of the most complex procedures of the external body. (Ciorba et al. 2006, p. 1508; Jessop et al. 2016, p. 2; Sivayoham et al. 2012, p. 288-290)**.**

3.3 Creating an Auricular Cartilage

There are many steps that lead to the reconstruction of an ear. First of all, tissue-specific cells (chondrocytes) need to be identified and harvested from the body in a biopsy (Ude et al. 2018, p.2). In addition, a scaffold in the shape of an auricle has to be produced in which the cultured chondrocytes, which are grown in a bioreactor, are seeded (Jessop et al. 2016, p. 4). These chondrocytes are grown in vitro within the scaffold and after time, the newly created auricle can be transplanted surgically. Scaffolds are important for stabilizing the tissue development in vivo and providing short-term mechanical stability (Ciorba et al. 2006, p. 1510) in order for it to be also replaced by the body's own cells over time (see figure 3) (Patel et al. 2012, p. 1128). Alongside the cultured cells and the biomaterial for a scaffold, a CT scan from the patients head is made, in order for an exact image of the auricle to be shot. Biomolecules are then united in vitro before everything is functionally integrated in vivo (see figure 4).

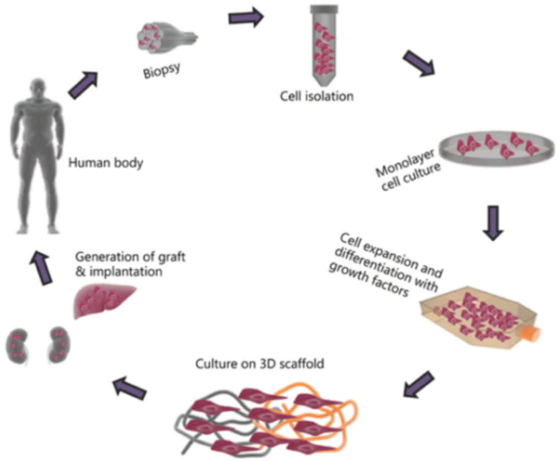

Figure 3*: The basic materials and processes needed for tissue engineering technologies*

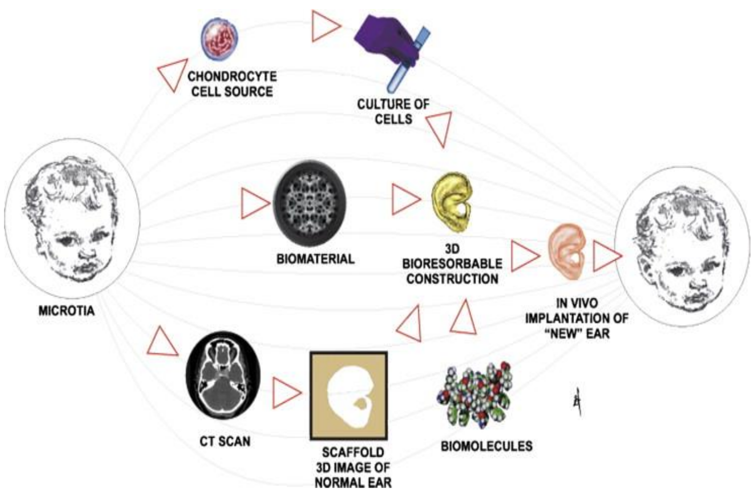

Figure 4: The main building blocks of auricular cartilage reconstruction

3.4 Cells

Due to the fact that the right cells are essential for the reconstruction of an auricle, a short input concerning the cells is given in this paper as well, however, only human chondrocytes and stem cells will be explained in detail (see figure 5). In addition, other factors for a successful auricular cartilage reconstruction will be presented in the following section.

1. Human chondrocytes
-Costal
-Auricular
-Articular
-Nasoseptal
2. Stem cells
-Embryonic
-Mesenchymal
-Umbilical

Figure 5: Cells used for auricular cartilage reconstruction.

3.4.1 Human Chondrocytes

Human chondrocytes are an important cell source for the reconstruction of auricular cartilages. Nevertheless, it is not only important to know which kind of chondrocyte cells should be used, but also from where and whom they are taken. Ideally, the patient's auricular autologous chondrocytes are taken from the originally damaged auricle but in severe cases of microtia, this is not possible (Patel et al 2012, p. 1124). Therefore, in most cases, costal cartilage is used (Weerdar et al. 2005, p. 381; Sivayoham et al. 2012, p. 288) because even today, it is seen as the only human chondrocyte source of quality with adequate quantity and acceptable morbidity (vgl. Ciorba et al. 2006, S. 1508). The harvest of costal cartilage, however, leads to donor-side morbidity, impedes the possibilities for shaping it, thus, results are often variable (Ciorba et al. 2006, p. 1509; Pintanguy et al. 2009, p. 447). Nonetheless, costal cartilage is not the only source of autologous chondrocytes. Articular, and naso-septal chondrocytes have been used as well (see appendix 9.1). It is important to understand that in recent times, progress has been made but it is still not possible to maintain characteristics like flexibility, strength and elasticity of the cartilage after insertion for a longer period of time (see appendix 9.2) (Jessop et al. 2016, p. 4; Upton et al. 1999, p. 302; Ciorba et al. 2006, p. 1508).

3.4.2 Stem Cells

For a long time, autologous chondrocytes were the main source for auricular cartilage engineering and chondrogenic stem cells have not been used for auricular reconstruction (Patel et al. 2012, S. 1124). It became clear from an interview with I.M. that chondrogenic stem cells seem to be a promising cell source for tissue engineering (I. M. 2020, Interview). These types of stem cells can either be taken from embryonic, mesenchymal (MSC) or umbilical stem cells (see appendix 9.3) (Ciorba et al. 2006, S. 1510).

3.5 Scaffolds

As mentioned earlier, the scaffold is essential for the reconstruction of an auricle. It needs to provide a three-dimensional environment to maintain the phenotype and topography to which chondrocytes can attach and later on differentiate and proliferate (Patel et al. 2012, p. 1128). In vitro, chondrogenesis also has to take place as well as in vivo the functional integration (Pitanguy et al. 2009, p. 448). One distinguishes between natural and synthetic materials.
Natural polymers have a specialized surface chemistry which is helpful for seeded chondrocytes because these surface characteristics stimulate cell adhesion and proliferation (Armiento et al. 2018, p. 1f). Nonetheless, weaknesses are a low mechanical strength of the natural scaffold and a fast degradation (Patel et al. 2012, p. 1129).

Apples only after being processed contain cellulose, a material that can be produced in high quantities (I.M. 2020, Interview). However, neither the amount nor the source of cellulose makes a proper cellulose scaffold but the way it is further processed is crucial (I.M. 2020, Interview; Osario et al. 2019, p.697f). The goal is to generate an optimized material that interacts with the used cell type. The properties of the scaffolds which are engineered for chondrocytes may be different from those which are made to grow human skin because the microstructure, topography and overall surface modifications of cellulose-based materials can control the interaction with cells and the development of the tissue (I.M. 2020, Interview). There are also some studies which show that scaffolds can be produced by bacterial cellulose with the help of 3D-printing (Wang et al. 2019, p. 4248)

Polyethylene is the most popular synthetic material used for scaffolds because of its flexibility, stability and simplicity to shape. Synthetic scaffolds are custom-made which is an advantage over natural materials. However, they can cause foreign body reactions due to degradation products and the lack of surface characteristics for optimal cell-attachment and growth (Patel et al. 2012, p. 1133).

The quality of the produced cartilage is influenced by the scaffold which may regulate the seeded cell's yield rate (Ciorba et al. 2006, p. 1512). Overall, an optimal scaffold has not been found yet. The question, whether the apple as a scaffold is a satisfactory solution, still remains.

3.6 Biological Factors

Not only the proper cells and scaffold have to be chosen but also the right biomaterial for the optimal growth of an auricular neo-cartilage (see appendix 9.4).

4. Interview

The interview was carried out on the 13th of March at the University of Basel in the department for biomedicine and had a total duration of an hour. Our interviewee was Prof. Dr. I.M, the group leader of the biomedical department at the University of Basel. With his research group, he focuses on understanding and applying the concepts of tissue engineering specifically for the knee cartilage. We had to condense the information efficiently in a compact way and consequently we only included the relevant questions and answers in the following section. The full interview can be found in the appendix (see 9.5).

Is creating an auricle out of an apple really that easy?

"It could definitely work, and it is a smart idea to use a natural material to generate a scaffold. Scientists do not use the fruit to generate the tissue, but as a scaffold to seed the cells that will later generate the tissue. However, the problem today is not having the proper scaffold, but having the proper cells that will generate the tissue on the scaffold. We have a wide variety of natural materials, synthetic polymers, even tissue derived materials, processed in different ways to have a variety of scaffolds on which the cells can be loaded. The problem is: Which are the good cells to be loaded? How can one control that the cells will properly generate a tissue once they are loaded? How long should one culture the cells onto this scaffold? When is the product ready to be implanted? How can one make everything well protected and resurfaced by skin? So, these are some questions for which there are currently many attempts to answer but yet no consolidated solution. I think the scaffold on which to grow the cells is a great idea, but I don't think it's the relevant part that will revolutionize the field because there are already many other solutions. It's

not the scaffold, it's the biology ultimately. One can argument with: "Creating an ear with an apple might be cheaper than with other materials." But if I think of the costs of taking the cells from a person and expanding them, bringing them into a scaffold and culturing them there, and the cost of a surgery to place the implant back into the patient with the proper follow-up, we are talking about thousands and thousands of Swiss Francs. And whether the scaffold costs one franc or one-hundred francs, is peanuts."

So, it would be even more difficult with an apple?
"Exactly. I'd say one of the weak points of this approach is a non-standardization of the material."

Which kinds of fruits and vegetables would be most suitable?
"There are already some materials, which are used clinically and are made out of cellulose, which is the main component of many fruits. But in this case, the materials which are made of cellulose, are made out of bacterial cellulose. You basically include the gene of cellulose in the genome of the bacteria and then you include a promoter which is active so that these bacteria will start producing a lot of cellulose. And this is a material which is produced in high quantities in bioreactors, it can be purified. And out of this bacterial cellulose, you can make all the possible scaffolds and materials you want. The only difference is the natural cellulose instead of this bacterial kind."

5. Discussion

In this part of the paper, our research is put together and the advantages and disadvantages of auricular cartilage tissue engineering and the creation of an auricle from an apple will be discussed. Furthermore, our last key question will be answered.

5.1 Advantages and Disadvantages

A benefit of using apples as a scaffold is that there are no ethnical concerns because apples are a neutral product which do not bring any harm with them. With collagen and sponges, however, which are alternatives to scaffolds made of apples, ethical questions and concerns may arise since they derive from animals. Specifically, the lives of sponges are put to an end for the creation of a scaffold (see appendix 9.5).

As a matter of fact, it is a smart idea to use natural materials like apples for the production of a scaffold because they are said to have suitable surface chemistry for cell adhesion and proliferation (Ivan Martin 2020, Interview; Armiento et al 2018, p. 1f). Generally, however, this cannot be said because not only the source is of importance but also the further processes under controlled conditions.

Additionally, the pulp of the apple could be made stronger by cross-linking as well getting chemically treated in order for it to be used as a scaffold (I.M. 2020, Interview).
It is also uncertain whether the flexibility of the scaffold can be maintained. In addition, the cellulose of which the apple is made of still has to be studied because it is yet to be understood how it should be processed in order for the properties of the scaffold to be improved and an optimal growth of a new tissue to take place.

A challenge with using an apple as a scaffold is that each one shows differences in density which makes the production of every apple scaffold more complex and less controllable. (I.M. 2020, Interview). Therefore, every apple would need to be inspected whereas synthetic scaffolds and even some natural ones which are for instance made up of swine collagen would not be as problematic. Thus, synthetic scaffolds allow certain degree of control in comparison to natural materials such as an apple.

Figure 6: Apples shaped in the form of an auricle.

Furthermore, the reconstructed auricle has to fit perfectly in order to be implanted. This may lead to another issue because it is almost impossible for mankind to carve the precise shape of an auricle out of the apple for every patient like Pelling did (see figure 7). Therefore, 3D-printed scaffolds are more precise than the less practical apples which could lead to severe problems after implantation like aesthetically undesirable consequences.

In his presentation, Pelling mentioned that costs for the production of a new auricle would be lower, if the scaffold would be made of an apple, which is correct. However, it is necessary to note that a biopsy costs a great sum of money as well as their expansion, seeding them in a scaffold and implanting them surgically. The production of a new auricle is expensive because almost all steps need to be completed under special conditions in clean rooms which are pricey to maintain. Consequently, the few hundred Swiss francs saved by a scaffold made with an apple instead of more expensive materials are only of little importance (I. M. 2020, Interview).

Scientists would also have to find out how an apple scaffold would incorporate with the seeded cells. The apple is a non-standardized material and no examination concerning its interaction with human chondrocytes, its biocompatibility and structural features for cell attachment, proliferation, and chondrogenesis in vitro and functional integration in vitro have not been studied yet (I.M. 2020, Interview).

Lastly, Pelling used mammalian cells instead of human chondrocytes or stem cells for his attempt to produce an auricle. It became clear from an interview with I. M. that with every kind of chondrocyte, fantastic tissues can be grown, even with mammalian chondrocytes. I.M. added that with chondrocytes taken from humans, a new set of challenges may appear because working with human chondrocytes is much more demanding (I. M. 2020, Interview).

Ultimately, the key to make auricular cartilage reconstruction more successful in the future, is to find the proper cells and understand how use them. Because only if the biology behind the process of the reconstruction of an auricle is better understood, the possibilities for the implantation of an auricle will improve (I.M. 2020, Interview). To give an example, a study from Zhou et al. reconstructed auricular cartilage surgically (Zhou et al. 2018, p. 287f) and showed that it is still unknown why in some cases the implanted auricle looked great whereas in other cases it did not. This is the reason why in this paper, a more detailed description of the different cell types was given (see appendix 9.1). Stem cells seem to be the cells source for the future, despite the fact that this has not been examined thoroughly yet (Terada et al. 2005, p. 962). The reason for that is because they are undifferentiated which makes them unique but also their ability for fast expansion and the low number of cells needed in the beginning for culture makes them a promising cells source. Costal cartilage, however, does not seem to be an ideal cell source in the future for auricular reconstruction because not only donor-side morbidity remains a grievous issue but also proliferation, dedifferentiation and the large number of cells for initial cell culture is too high.

5.2 Is this idea revolutionary?

It is in fact possible to use fruits as a scaffold for auricular reconstruction (I. M. 2020, Interview) Nevertheless, it has to be understood that the apple phenomenon only describes a possibility for a scaffold made by a cheap material and does not include a potential solution for the usage of the right cells type in auricular cartilage reconstruction. Consequently, this idea will not benefit the research for auricular reconstruction.

6. Summary

Scientists are attempting to find different ways to improve auricular cartilage tissue engineering. Andrew Pelling came up with the idea of using an apple as a scaffold by removing the apple's cells and DNA, however, it seems as if the idea of using an apple as a scaffold may be creative but even producing a scaffold that unites all components needed would only solve one of several problems to reconstruct an auricle because many more factors also influence the product, such as the proper cells, the culture duration, the culture conditions, the cost and much more. Thus, many different questions still remain that make auricular cartilage reconstruction challenging. There have been many attempts to solve these questions but according to I. M. these answers have not been found yet.

7.Conclusion

To date, it is unknown whether Pelling's idea will revolutionize auricular cartilage reconstruction because further studies need to be done. Hence, Pelling passed his discoveries and ideas off as a milestone in auricular cartilage reconstruction and let us believe with his positive and optimistic attitude that he found the solution with apples as a scaffold while ignoring the fact that the key for auricular reconstruction is more than just a scaffold. The idea of using an apple as a scaffold seems interesting, but so far, Pelling did not produce any solutions for the reconstruction of an auricle. So far, there are no real advantages apart from the ethnical reasons to use apples as scaffold because it is still uncertain whether it is suitable for a scaffold. Therefore, the disadvantages overweigh the advantages.

We were told that's why Andrew Pelling was not given attention among experts regarding auricular reconstruction. In conclusion, Pelling has not found the solution that would revolutionize auricular cartilage reconstruction but perhaps the purpose of his discovery was just being creative and reflective, while also giving us something to think about, instead of solving problems.

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Figure 7: Analynn Souvannavong: “Swine Collagen” (13/03/2020)

Figure 8: Analynn Souvannavong: “Clean Room Arrangement at the University of Basel“(13/03/2020)

9. Appendix

9.1 Different Human Chondrocyte Cell Types

It is said that naso-septal cells form a cartilage which consists of high collagen II-to-I ratio and proliferate at a faster rate than articular cartilage (Kafienah et al. 2002, p. 817). We learned from an interview with I.M. that cells form the naso-septal can be expanded and regenerate cartilage in a more efficient and reproducible way than articular chondrocytes and donor-side morbidity is minimal (I.M. 2020, Interview; Von Rechenberg et al. 2016, p. 1286).

Furthermore, Jessop et al. explained that the use of articular, costal and naso-septal chondrocytes does not result in the production of elastin containing cartilage which is of importance for the production of an auricle (Jessop et al. 2016, p. 6).

9.2 Benefits and Issues of IHC

There are some benefits of using costal cartilage. The lack of antigenicity enables long-term stability (Lin et al. 2018, p. 168) and once the implanted ear is healed, no further treatments would have to be done ever again (Siegert et al. 2009, p. 169).

Nevertheless, isolated human chondrocytes are challenging due to proliferation, dedifferentiation and large amounts of cells needed for neo-cartilages (Verhaar et al. 2004, p. 109) because after extension, they change their properties (I.M. 2020, Interview). The growth of chondrocytes in number and proliferation makes them lose their functionality. That loss of functionality is not necessarily bad, as long as these cells are able to regain it (I.M. 2020, Interview). In addition, the dedifferentiation process leads to a change from collagen type II to collagen type I synthesis and to a loss of the phenotype in monolayer culture, so they turn into fibroblasts (Ciorba et al. 2006, p. 1511; Patel et al. 2012, p. 1128). It has to be mentioned that the large number of cells needed for neo-cartilage (Verhaar et al. 2004, p. 109) and the expansion of these cells in a short period of time are further weaknesses of using autologous chondrocytes (Patel et al. 2012, p. 1125). Some other issues like long-term limitations have emerged in vivo. Firstly, the reconstructed cartilage auricle may become resorbed as well as lead to extrusion which causes a loss of protection (Jessop et al. 2016, p. 3). Secondly, stiffness of the implanted auricle and distortion are problems as well (Jessop et al. 2016, p. 3). Thirdly, neo-cartilage is often prone to calcification and therefore easily breakable (Jessop et al. 2016, p. 4; Landis et al. 2009, p.136f.). Lastly, immature cartilage is highly active metabolically as it grown and develops. Nonetheless, it has not been found out yet what is needed to mature cartilage (Jessop et al. 2016, p. 6).

9.3 Stem Cells

Embryonic stem cells are not used for auricular cartilage reconstruction due to ethical reasons (Patel 2012, p. 1125). Consequently, the focus in research lies on adult stem cells with the most promising source being the bone marrow where MSC is found. In the human bone marrow, two types of stem cells are found: HSC and MSC (Pitanguy et al. 2009, p. 448). However, only MSC is used because they show chondrogenic potential (Chen et al. 2003, p.274). MSC are easy to obtain and show an extensive replication in vivo and in vitro which is exceptional and in comparison, to autologous chondrocytes, only a low number of cells are needed for initial culture (Ciorba et al. 2006, p. 1511). Umbilical stem cells also show chondrogenic potential, however, more studies need to be done (Terada et al. 2005, p. 962). It has to be mentioned that stem cells do not tend to redifferentiate, once differentiated (Patel et al. 2012, p. 1125). Moreover, "*the challenge in culturing stem cells is directing it in a desired differentiation pathway through specific biomechanical mechanisms, which is not a fully understood process. There is a risk of indefinite proliferation and a growth of a tumor." (Patel et al. 2012, p. 1125-1126).*

9.4 Biological Factors

"Essentials including growth factors have been used to further induce chondrocyte growth and accelerate cartilage formation, including growth factors and bioreactors." (Patel et al. 2012, p. 1128). Growth factors may directly be put into the scaffold or added in culture media. Therefore, chondrocyte differentiation and the promotor tissue formation can be controlled (Patel et al. 2012, p. 1128). *"Biomaterials are used to guide the organization, growth and differentiation of cells in the process of forming functional tissue and can provide both physical and chemical cues." (Pitanguy et al. 2009, p. 449).*

9.5 Full interview

What is regenerative medicine all about?

"Regenerative medicine historically evolved from initial concepts of tissue engineering. Scientists were envisioning the possibility of building in vitro body parts. They thought it can revolutionize tissue therapy and tissue reconstruction, so in the 1990s, a lot of progress has been made in generating body parts ex vivo, especially for tissues for which it was easier to control the biology of the cells, such as cartilages and bones. Later, there was a clear recognition that this would not be sufficient. Because having even the best possible graft for implantation might not imply that it would be functional. It would have to connect properly with the surrounding environment. In some cases, it is related to the interphase with soft tissues. It should be seen as an integrating part by our inflammatory cells, which are critical even in the smallest possible cut in your hand and that initiate a cascade of processes that can lead to the regeneration of a small cut but in some cases prevent the regeneration. So, here is where historically people recognized, it is not only about engineering a tissue, but also about understanding in a broader sense, what are the regenerative processes which are required to better control them once the graft is implanted and maybe it's sufficient to deliver the signals in our body that make our own cells regenerate a specific part of our body. So, this is the evolution of a concept from tissue engineering to regenerative medicine. Nowadays if you speak about regenerative medicine, you have a very broad set of possibilities, one of which is based on engineering a tissue outside of our body and then implanting it, but many of the other approaches are related to the design of smart materials or drug delivery systems that can induce the own body to regenerate a part. It is all about understanding regenerative processes and learning how to control them with e.g. smart materials and drugs as well as signals like growth

factors, drugs, pills that can be delivered locally and that induce a certain set of reactions or that control reactions that would occur anyways."

Is creating an ear out of an apple really that easy?
"It could definitely work. It is a smart idea to use a natural material to generate a scaffold. Scientists do not use the fruit to generate the tissue, but as a scaffold to seed the cells that would generate the tissue. There are ways to make the pulp of this apple strong enough by cross linking and processing it chemically, so that it could effectively function as a scaffold for the growth of a tissue. However, the problem today is not having the proper scaffold, but having the proper cells that will generate the tissue on the scaffold. We have a wide variety of natural materials, synthetic polymers, even tissue derived materials, processed in different ways to have a variety of scaffolds on which the cells can be loaded. The problem is: Which are the good cells to be loaded? How can one control that the cells will properly generate a tissue once they are loaded? How long should one culture the cells onto this scaffold? When is the product ready to be implanted? How can one make everything well protected and resurfaced by skin? So, these are some questions for which there are currently many attempts to answer but yet no consolidated solution. I think the scaffold on which to grow the cells is a great idea, but I don't think it's the relevant part that will revolutionize the field because there are already many other solutions. One can argument with: "Creating an ear with an apple might be cheaper than with other materials." But if I think of the costs of taking the cells from a person and expanding them, bringing them into a scaffold and culturing them there, and the cost of a surgery to place the implant back into the patient with the proper follow-up, we are talking about thousands and thousands of Swiss Francs. And whether the scaffold costs one franc or one-hundred francs, is peanuts."

What would the alternative materials be for creating a scaffold?
"The materials we use as a scaffold to generate tissue which is later implanted in patients are, for example sponges made from collagen. Collagen is a very abundant material. The problem here is not the cost of the collagen itself, but the processing of the collagen, in order for it to reach a clinical grade and to make it compatible with safety and standardization to bring it to a patient. We, for instance, use membranes out of collagen from a swine. It has been processed in different ways and is therefore very thin. Or here, we have the cocoon from a silkworm and with it, it's possible to isolate and purify threads of silk for it to get processed into a tissue and ultimately use it as a scaffold. It is highly biocompatible, resistant and one can control the degradation rate. So, these are some examples of natural polymers or natural materials. And then you have all the classes of synthetic polymers such as polyglycolic acid and polycaprolactone. These materials give you the possibility of applying the 3D printing technology in order to generate a structure with a precise size and shape and the architecture of the pores, layer by layer."

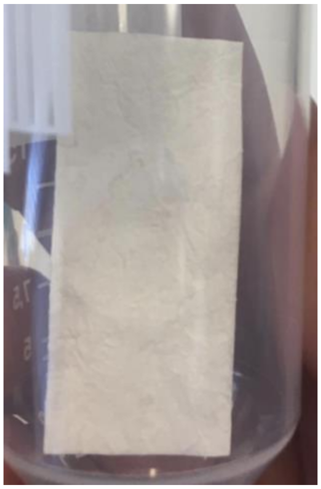

Figure 7 Swine Collagen

So, it would be even more difficult with an apple?
"Exactly. I'd say one of the weak points of this approach is a non-standardization of the material. It's not the scaffold, it's the biology ultimately."

Which kinds of fruits and vegetables would be most suitable?
"There are already some materials, which are used clinically and are made out of cellulose. Cellulose is the main component of many fruits. But in this case, the materials which are made of

cellulose, are made out of bacterial cellulose. You basically include the gene of cellulose in the genome of the bacteria and then you include a promoter which is active so that these bacteria will start producing a lot of cellulose. And this is a material which is produced in high quantities in bioreactors and it can be purified. And out of this bacterial cellulose, you can make all the possible scaffolds and materials you want. The only difference is the natural cellulose instead of this bacterial kind."

What are the steps for making an ear out of an apple?

1. *"We need the right source of the cells: So, for example, if we are interested in replacing the articular cartilage, we do the most obvious thing, which is to take cells from pieces of the articular cartilage. By doing that we have seen that it is extremely challenging because you have to take a small biopsy.*

2. *The goal is then to expand and generate a tissue which is bigger than the original biopsy, otherwise there would be no benefit. Once you expand these cells, which are called chondrocytes (so, the cartilage cells), they change their properties. The induction to proliferate makes them lose their functionality. And that's not bad as long as they can regain their functionality. We found out that the cells are good at regaining their functionality once they are placed in a 3D environment. But in some cases, it doesn't lead to the same result. However, we found out that the cells of the nasal septum can be expanded and have the capacity to regenerate cartilage in a more efficient and reproducible way. We are now trying to understand how nasal cartilage cells differ from the articular cartilage cells and what the genetic or epigenetic features of them are, so this is still a bit of a mystery. But in the meantime, it works. So now we are using nasal cartilage cells to grow cartilage for the knee.*

3. *Now we have the cells to put into the material. The next challenge is understanding the culture conditions. If you grow the cells on a specific material, they need a couple of factors to support them, which are typically taken from the serum of bovine material, because it is very rich in proteins. But if we want to put the implant back into the patient, we cannot use bovine proteins because they could remain in the tissue which is being generated. Once implanted, they can induce an immune reaction and this graft can induce a strong inflammation. So, we had to look for alternatives for this bovine serum that would enable the generation of a tissue which is compatible with a human implantation.*

4. *Then you have to discuss which materials to use, which regions are suitable to grow the tissue and so on. Associated with it is also the time because it is extremely expensive to grow cells in clean rooms. Additionally, you have to dress in a sterile way and there is also limited space.*

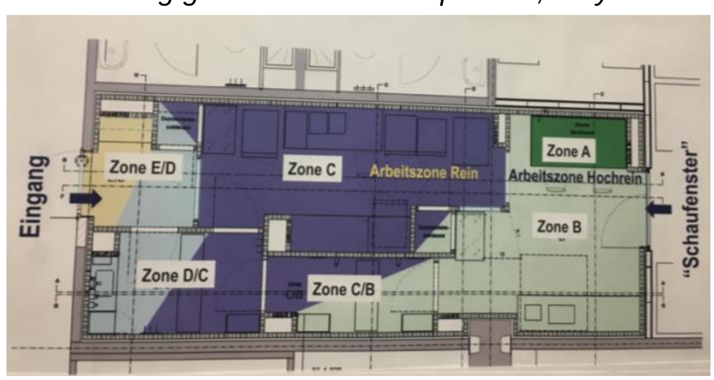

Figure 8 Clean Room Arrangement at the University of Basel

5. *Next problem: Which surgical procedure should you use, and which type of patient will benefit from it? You should also ask yourself what you should ask the patient to do. Should they walk or avoid walking? Some are convinced that the key to success or failure is the type of rehabilitation the patient receives. Every patient needs a different kind of fixation: Should you press fit the cartilage? Does it need sewing? Should you apply biological glues? Even with the best possible graft, if you do not have the right type of rehabilitation that is matched with the right type of defect you have, the whole thing can fail or succeed. If it works for individuals, we have to demonstrate that this is reproducible with many other people in order for insurances to pay for it. Otherwise no company wants to commit to produce it and then there is also no offer. So, the economic costs and efficacy aspects are also critical.*

And then there are even more questions that are still left to answer:
What cells should one use to recolonize an apple to make it into a piece of cartilage? How long should it be cultured? What is the surgical procedure? How long should it remain protected? When can it be exposed? And the list goes on.
There are recent studies by Chinese groups that effectively engineered ears and applied them in children Ultimately the outcome of the study was that they don't know which procedure is best. And following this, no health insurance would reimburse this."

How would you connect this topic to genetic engineering?

"Here, there is no genetic alteration of the cells. However, there are certainly groups that are convinced, that you would have to program (genetically manipulate or correct) the cells in order to have a more efficient regeneration. Because they come from adults that have limited capacity."

Regenerative medicine is a relatively new medical field. What is the meaning of it mainly for the future?

"The major breakthrough that I think the field of regenerative medicine has to make, is to better understand the biological processes of regeneration. In other words, going back to the fundamental science and not the clinical application. So, what happens in our body when you implant a certain type of cells, a certain type of tissue and what the reactions are that occur from the cells of our own body because only if we understand this, we can better control it. And we know so little. It just starts to be understood that populations of blood cells, lymphocytes, can participate to decide whether a regeneration will take place or not. Earlier, I was making an example with a cut, so here the cells which play a role are known for many years. So, first there is a wave of neutrophils that comes and then this attracts monocytes, macrophages and this attracts blood vessels and skin progenitors. And still investigating deeper, we realize that we do not know everything. There is a lot of research currently on how the mechanical properties of material can determine whether the whole process will be successful or not (whether the cells will recognize it as nice and friendly).
The hard part is the variability in the preparation of the different batches. This would the challenging part in creating those scaffolds."

9.6 Glossary

Word	Meaning	Page number
antigenicity	*"Any substance that can stimulate the production of antibodies and combine specifically with them."*	12
articular	*"Something Referring to the joint."*	12,15
auricular	*"Relating to the external part of the ear."*	1,2,3,4,7,8,13
autologous	*"Taken from an individual's own tissues, cells, or DNA."*	4,12,13
chondrogenesis	*"the development of cartilage"*	4,7
collagen	*"A type of protein fiber found abundantly throughout our body. It provides strength and cushioning to many different areas of the body, including the skin. More specifically, collagen is found in our various types of connective tissues such as cartilage."*	3,6,7,12,14
costal	*"Something relating to the ribs."*	4,8,12
differentiation (to differentiate)	*"The normal process by which a less specialized cell develops or matures to become more distinct in form and function."* **and „de-differentiation"?**	8,12,13
elastin	*"A protein that is similar to collagen and is the chief constituent of elastic fibers."*	12
extracellular matrix	"The non-cellular portion of a tissue produced and secreted by cells and mainly for providing support."	3
fibroblast	*"A cell that produces collagen."*	12
insertion	*"The act of putting something inside something else or adding something."*	4
mesenchymal (MSC)	*"Referring to cells that develop into connective tissue, blood vessels, and lymphatic tissue."*	4
microtia	*"abnormal smallness of the external ear"*	4
naso-septal	*"Something related to the internasal septum, a cartilage within the nose."*	4,12
proliferation (to proliferate)	*"The process that results in an increase of number of cells and is defined by the balance between cell division and cell loss through cell death of differentiation."*	4,6,7,8,12,13
to redifferentiate	*"A process by which a group of once differentiated cells return to their original specialized form."*	13
umbilical	*"of, relating to, or used at the navel"*	4,13

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