Biology Term Paper
Genetics
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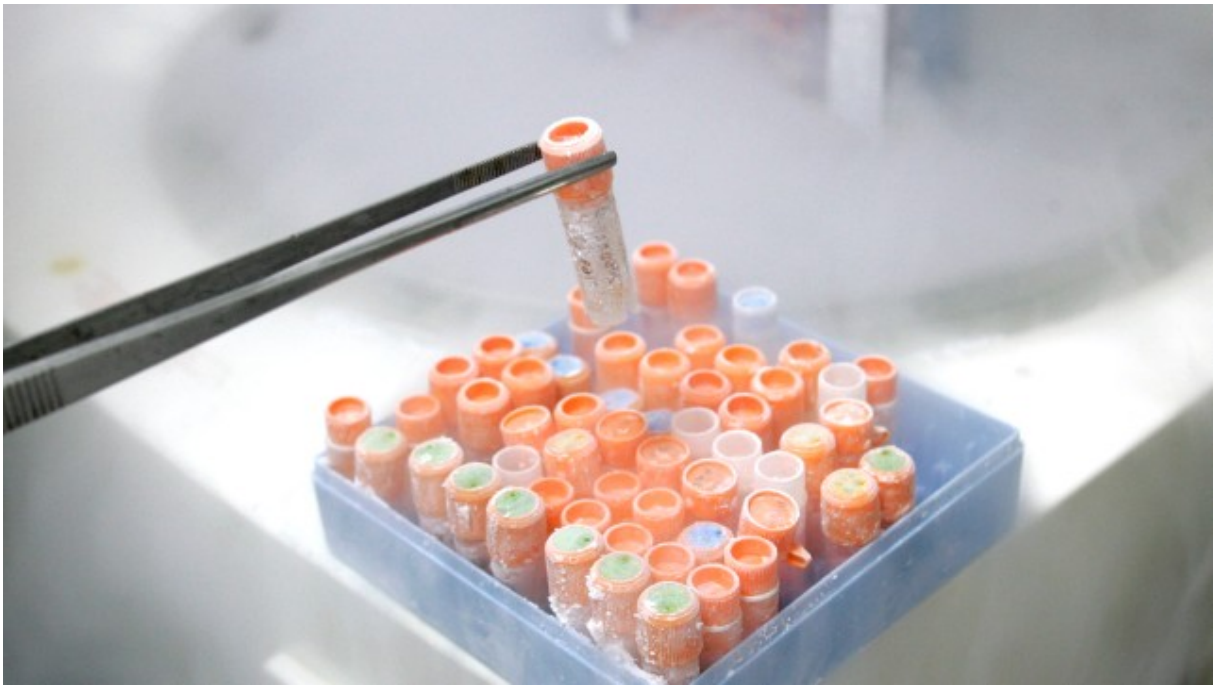
Gene Therapy

A paper about the application, mechanics and effects of controversial approaches in the field of genetic engineering



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Robin Anyanwu
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Introduction

Our topic can be divided into two therapies. The cell and the gene therapy. The cell therapy focuses on changing a cell in a cell population to treat a disease. The gene therapy on the other hand changes a gene, which then gets put into a cell in the body to treat the disease. You can combine these two methods. This is called the cell and gene therapy approach.
The research group we visited is working in the laboratory and they are trying to find new ways to treat diseases with cell and gene therapy. They are focusing on finding a way to treat ischemia, a disease where the blood flow isn't working correctly and the body is missing oxygen and glucose.
Some of these therapies are commonly used in medicine but others are experimental because for example the success with this technique is not constant enough so you can't use it with patients. The technique first has to pass test with animals.
With the help of these two therapies it can be possible to cure diseases which at the moment aren't curable but this is in far future because if they are able to find a technique which can treat such a disease it has to go through several test phases before it can be frequently used in a hospital for example.
Because these kinds of treatments aren't used so frequently it is even more fascinating when it works and a patient with a disease gets treated and actually cured forever. In fact such a success is the work of years of research and tests and the goal is of course for those treatments to be accepted and to cure as many patients as possible.
This is also the goal of the research group we visited at the University of Basel.

Preface

When we were looking up a topic for our term paper the topic of gene therapy really caught our interest. We are all three very enthusiastic when it comes to experimental and laboratory work in biology. Therefore we were really motivated to find out more about this research that uses among medically accepted techniques also highly experimental and new techniques to cure diseases, which are commonly known as incurable.
We find it very interesting that it is in fact possible to for example regenerate blood vessels to help regenerate blood flow and through that treat ischemic diseases or improve vascularization in tissues. This of course opens new and better possibilities to cure certain diseases like for example ischemia. (Restricted blood supply to tissues causing a lack of oxygen and glucose. This can lead to the death of the tissue).
So we want to find out what big achievements the research has made until this day. This we will hopefully accomplish with our visit at the University of Basel. When we are there we also want to know better how the research group works and maybe we will get to see a laboratory. In general we just want to find out how the gene therapy works in detail, how much knowledge has been achieved through this therapy and how the work in a research laboratory looks like.

A short overview of the paper;

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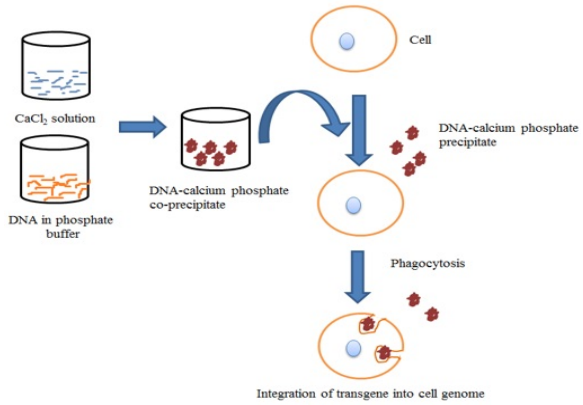
Description of engineering techniques

The goal of gene therapy is adding a gene into a cell in order to replace a gene that was not working properly before, which as a result will cure a certain disease.
In this section, we will explain the applied methods, also called vectors, of gene therapy used to insert DNA inside a cell.

Transfection

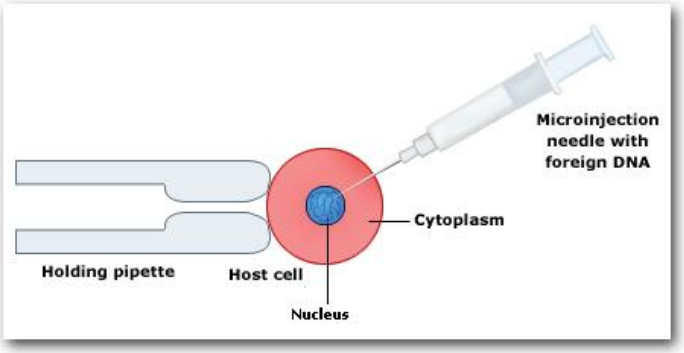
Calcium phosphate precipitation

The therapeutic gene and a polyatomic ion (f.e calcium phosphate) are added to a cell. The polyatomic ion has the ability to disturb the structure of the cell membrane, causing endocytosis. Endocytosis is the absorption of unfamiliar cell material through invagination of the cell membrane. This means that the DNA can get into the cell during this time. However, the chance that the DNA is installed successfully is 1:1000 to 1:100000. There is a really low possibility that the installation could work.



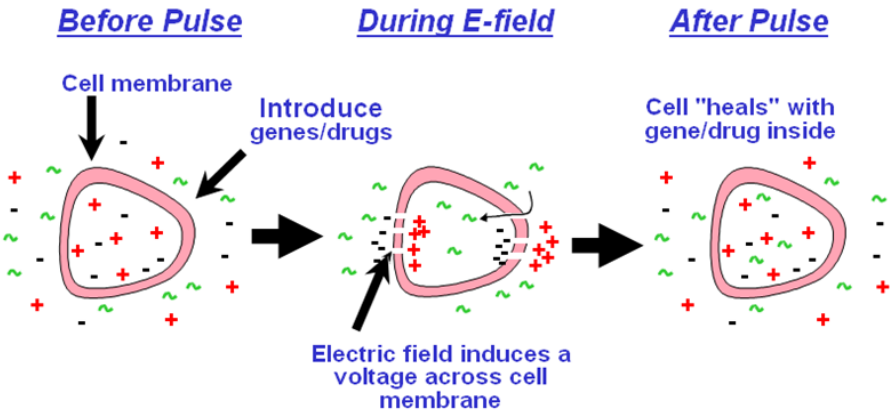
Microinjection

Another procedure is the microinjection. The DNA(in form of a plasmid) is injected directly inside the nucleus with the help of a micro capillary. The chance that the DNA is injected successfully is 1:5.This is a much higher chance than in the former method. The disadvantage although is that every single cell must be treated separately. This is very time consuming, as it is only possible to do this 60-200times an hour.



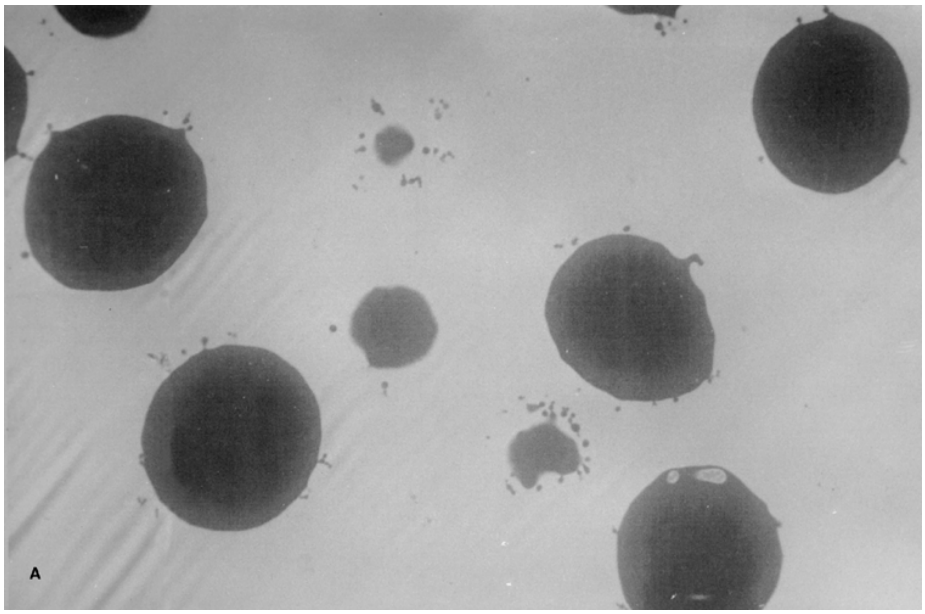
Electroporation

The third procedure we look at is the electroporation. In this method voltage pulses make the cell membrane of a cell permeable temporarily, so DNA can enter the cell.
This method is quite inefficient, because the cell can be damaged badly.



Erythrocyte ghost

The last transfection method is the erythrocyte ghost. First the erythrocytes are brought to lysis in a solution containing the therapeutic gene. The cell membrane of the erythrocytes closes soon with the therapeutic gene capsuled in it.
Afterwards the erythrocytes are fused with the target cell.

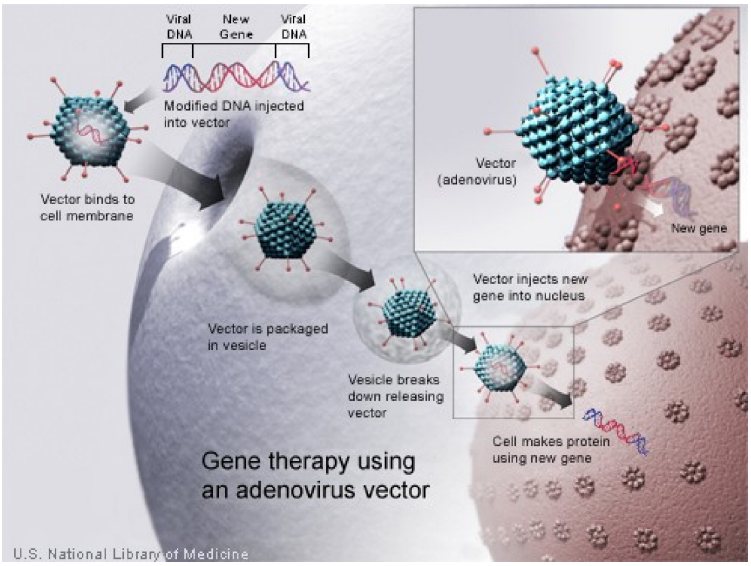


Transduction

In these procedures a transport virus is used to insert the therapeutic gene into the target cell. The viruses used in gene therapy can not harm the organism and have the ability to spread. Therefore the virus must be modified before the use of gene therapy.

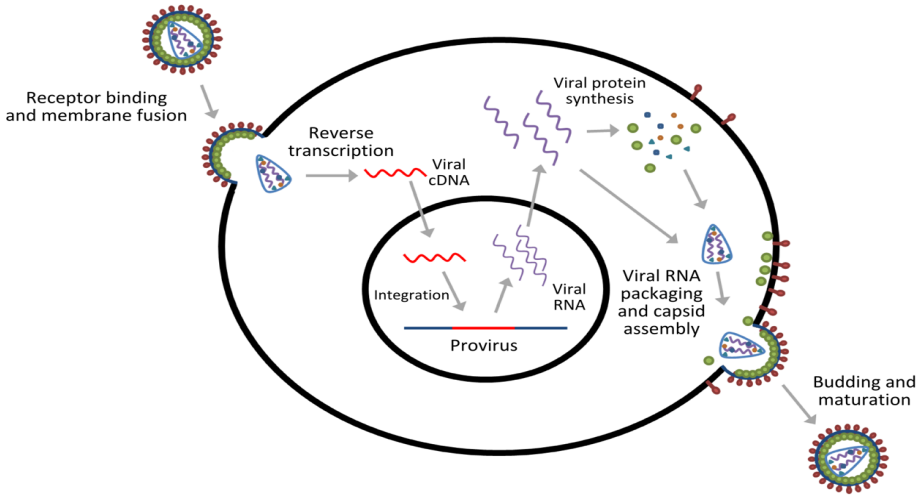
DNA viruses

These viruses spread by injecting their DNA in a host cell. The therapeutic gene can be inserted into the genome of the virus. The virus penetrates the cell membrane and releases its DNA in the cell, containing the therapeutic gene. In comparison to other viruses the injection is easy, due to already having DNA. However, these type of viruses are only capable to penetrate a few cells.



Retro viruses

These viruses stick to the cell membrane and inject proteins able to do reverse transcriptase and its RNA. The therapeutic gene can be implemented in the genome, where the code for structure of the virus is found. Only the Y region of the virus is left to convert the DNA into RNA. The RNA can be transcribed to DNA in the cell, so leftover is the therapeutic gene. This type of virus is capable to infect many cells, making it one of the best transport viruses. A problem is that if it is able to spread can cause tumours.



Interview Dr. Andrea Banfi: Cell and Gene Therapy & Photos Laboratories Hebelstrasse 20; Zentrum für Lehre und Forschung

A short explanation/summary; what is cell and gene therapy?
First of all, a therapy is the development of a strategy to cure diseases.
In cell therapy the cell population is isolated from the patient or maybe a donor.
You can use these cells after manipulating it to treat a certain disease.
Gene therapy refers to the fact of changing the expression of some therapeutic gene.
so delivering a construct, which will put the gene inside cells of the body, so it corrects and treats the disease. It is possibly to combine the therapies by delivering cells that have been genetically modified in vitro, meaning in the lab. This combination is called the cell&gene therapy approach.

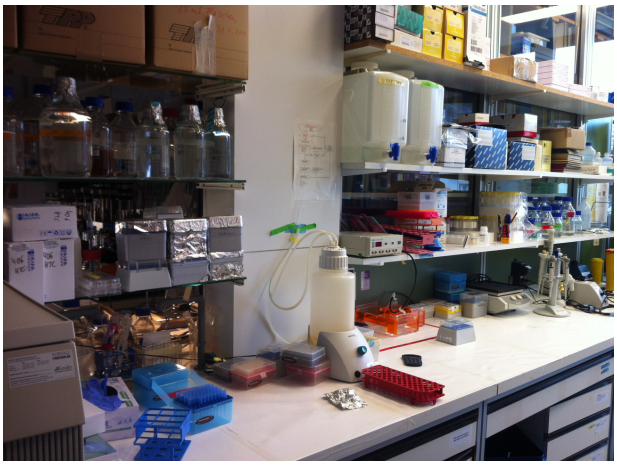
What is the goal of "The Cell and Gene therapy group"?
Our goal is to use cells, genes and modified cells to treat a specific kind of disease called ischemia. Ischemia is a situation where in the body blood flow is not enough.
As example take a heart attack. A heart attack is caused by an ischemia in the arteria, so it is also named heart ischemia. That is why we need to induce the growth of new blood vessels. On one hand we study the biology of vascular growth in order to understand the regulatory signals that are important and than use cells, modified cells or genes to go and affect those pathways, those signals inside the infected disease areas, so that new blood vessels can grow. On the other hand we look at the approach of tissue engineering. That is engineering of a piece of body. This relies on factor, having a structure. This structure is called a scaffold that has the shape, signals and puts the tissues together and the cells that need to be the right progenitors to produce that piece of tissue. These cells are the so called stem cells.
Let us take a bone replacement as an example. In this case you take a stem cell, put it on a scaffold and it will create the bone, so that you can use it to replace it for people having for example cancer in their bones. So in this because the tissue engineering of bone is something that you try to create the piece of bone to substitute what you need to take away, because of trauma, surgery, cancer and some other diseases.

What methods do you/ does your group apply to do research on this topic? Do you operate them on patients?
Some of the methods are used in clinics today. However, many others are still experimental, since not enough is known yet to actually achieve the goal in the patient. As an example of experimental method is the bone replacement example which is not possible yet.
There are results in the middle, results that suggest it could be possible, but it is not sufficiently effective yet to actually treat a patient. For this, you usually use hemopoyeticstem cells. (...)
They are the first stem cell that has been discovered, characterised and understood, and bone marrow transplantation is the first stem cell transplantation which is now no longer experimental, since 20 years it's just routine. You do bone marrow transplantation to treat a wide variety of diseases, from ischemia to genetic diseases where a certain enzyme is not efficient. Stem cells in a scaffold tissue engineering, this is happening; the group next door, the tissue engineering group, did a clinical trial to generate pieces of cartilage to repair a surgery to the nose for example. (...)
Genetically modified cells, the classic trials here on patients, is for immune disease. So these are the so called Bubble Boys. These boys lack one gene since birth, they basically cannot

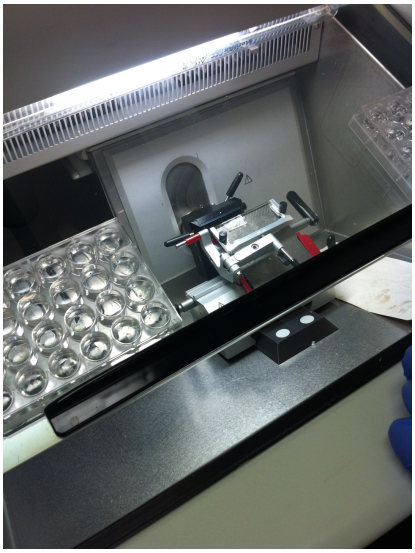
make a functioning immune system. They would normally die in short time by any kind of infection. So they need to live in a bubble, an enclosed environment that is completely sterile. It's a horrible life. So now you can take the stem cells of the boys, put a viral vector in there carrying the defective gene to bring the correct copy, by transplanting it back in vivo, it could generate a whole new working immune system. This treats the disease completely.
(...)
The boys who underwent these trials in the early 2000snds are still alive and well, except for three who developed a cancer and died. (...)

Concerning the cure of ischemic and vascular diseases, what progress were you able to achieve during your work?
We focus on blood vessel growth. When we started working on this, it was known what is the main signal that can tell blood vessels to multiply and grow into new ones. But when it was tried to use it in therapy, in the 90ies, it just didn't work. And when I started working on this, when I was doing my postdoc in Stanford, California, there was the moment in which clinical trials have been done and a couple years later, the results came out that it was negative. What our team tried to understand in the next 10 to 12 years was: Why did that factor not work? Is it the wrong factor or do we just don't know enough how to give it? And the answer is no it is the right factor, there is no other factor that is better, but you need to understand how it works. So we have now understood a large number of things that you can not just give the factor, you have to give it at controlled dose, controlled distribution in the tissue, so you need to develop ways to deliver the factor that are not just delivering a gene therapy director, but we have developed the material for scaffold, and then we can actually get hold of the growth in a therapeutic way. But all this of course is not in the clinics. Because you do a clinical trial based on the biological concepts of five years before. So it all takes a lot of time, new discoveries cannot be incorporated into the planned trial. (....) Also, you need to exactly work out the details for a planned treatment, therapy or experiment.
(...)

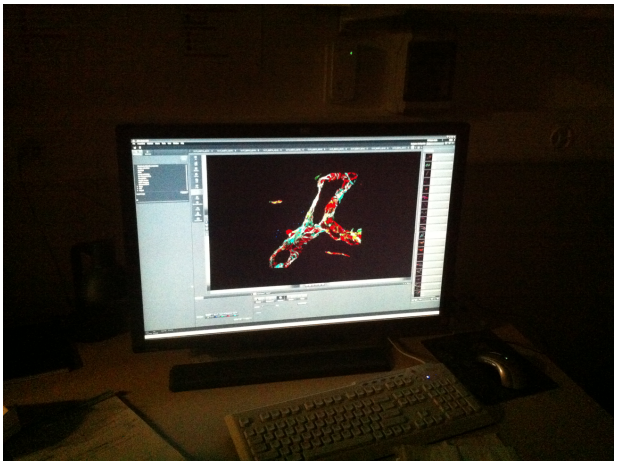
What do you think will the subject of stem cell/gene therapy develop like over the next few years? Will it be a successful way of treating diseases?
Well let the next generation say what they hope for the future!
(Male and female PhD doctorands are asked)
Woman: We hope that all this study will end up in some clinical approach, because now, for me for example, I'm studying both the molecular basis of angiogenesis but also I'm trying to develop a clinical therapy approach to treat ischemia so I hope that once I will end up in something that will help someone, hopefully.
Man: Imagine that if you discover something and then you can go into the clinic so the patient is treated tomorrow, it doesn't work like that. So you need to really investigate a lot especially like this basic mechanism like it is in biology, to understand that and then.
Woman:Like with the development of a drug, first you need the in vitro study, after you need to pass it in vivo, with animals.
Banfi: There is like a strategy which is designed to guarantee a certain safety to the patient, otherwise it would be uncontrolled. (...)
Woman: Sometimes it can also take 20 years or more...Its Years of Study.
Banfi: But the university of Basel is a good place in the world for this kind of research, for stem cells, for regenerative medicine. Its really a good place. There are very good results and good studies being carried out by a large number of groups.



Bits of the usual laboratory of the "Cell and Gene Therapy Group" at Hebelstrasse 20, Zentrum für Lehre und Forschung.



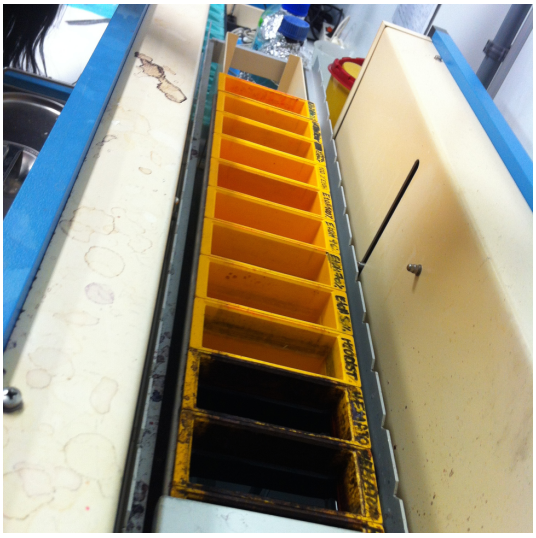
A device which cuts frozen tissue in extremely thin slices to be examined. (Here: Rat brain)



An electronic microscope + Software which colours the stained parts in tissue (e.g. cell nuclei, blood vessel growth etc.).

Freezer for tissue and muscle donations/examples, minus 80 degrees Celsius.

The rat brain from before is stained in various colours to identify different tissues.



Cartages for the colouring of the tissue. Different colours provide differentiation between the tissues.

Discussion

What progress was made with the application of the chosen technique?
Gene therapy is an experimental technique that uses genes to treat or prevent diseases. In the future, this technique could allow doctors to treat a disorder by inserting a gene into a patient's cells instead of using medicine or surgery. Scientists are currently testing several approaches to gene therapy, including:

- Replacing a mutated gene that causes disease with a healthy copy of the gene.
- Inactivating, or "knocking out," a mutated gene that is functioning improperly.
- Introducing a new gene into the body to help fight a disease.

Although gene therapy is a brighttreatment option for various diseases (including inherited disorders, some types of cancer, and certain viral infections), the technique maintains an overall risky aspect. But despite the negative effects, gene therapy could already now safe lives in human application.

What future research steps?
Current research is testing out the safety of gene therapy; future studies will find out whether it is an effective treatment option. Because the techniques are relatively new, some of the risks may be unpredictable; however, scientist and institutionsare working together to ensure that gene therapy research is as safe as possible. Researchers must overcome many technical challenges before gene therapy will be a practical approach to treating disease. For example, scientists must find better ways to deliver genes and target them to specific cells. They must also ensure that new genes can be controlled by the body.

Discussion of ethical aspects
Because gene therapy involves making changes to the body's basic state, it raises many ethical concerns. The questions surrounding gene therapy are as follows;

- How can "good" and "bad" uses of gene therapy be distinguished?
- Who decides which traits are normal and which indicate a disorder?
- Will the high costs of gene therapy make it available to everyone?
- Could the spread of gene therapy make society unaccepting of people who are different
- Should people be allowed to use gene therapy to pick basic human traits such as look or intelligence?

Current gene therapy research has focused on treating individuals by applying the therapy to body cells such as bone marrow or blood cells. This type of gene therapy cannot be passed on to the offspring. Gene therapy could be targeted to egg and sperm cell (germ cells), however, which would allow the inserted gene to be passed on to later generations. This approach is known as germline gene therapy.
Of course, the idea of germline gene therapy is controversial. While it could preserve future generations in a family from having a particular genetic disorder, it might affect the development of a fetus in unexpected ways or have long-term side effects that are not yet known. Because people who would be affected by germline gene therapy are not yet born, they can't choose whether to have the treatment. Because of these ethical concerns, the application of germline gene therapy is prohibited in more than 80 countries in the world.

Summary

Looking back to the start of our group work we have really learned a lot. We wanted to know more about the gene and cell therapy and the interview with Dr. Andrea Banfi has really helped a lot in that sense. In detail he told us about the general meaning of these therapies and how they exactly work, meaning that he told us about methods which apply the mentioned therapies. So we have learned about several examples of gene & cell therapy methods used to cure different diseases. Then the research group around Dr. Banfi has told us about their achievements in the past and about their future goals. They also explained to us that they are searching for a way to treat ischemic and vascular diseases. They showed us around in their laboratory and we were able to see things we've only heard about in class with our own eyes which was a great experience. Furthermore we've dealt with different aspects of the gene therapy like f.e the ethical aspects and also the difficulties these methods have to acclimatize themselves in the modern medicine.

To conclude we've certainly reached our goal and learned a lot about cell & gene therapy. We've got a taste of the experimental laboratory work which was an unforgettable experience. The whole work on this term paper was very interesting and informative. We would like to thank the research group around Dr. Banfi from the University of Basel for having us at the institute and for being a great source of information for our term paper.

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