

dpte

Dutch Program for Tissue Engineering

DPTe Gathering
the fruits of
exploration
and collaboration

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the fruits of
exploration
and collaboration

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Introduction

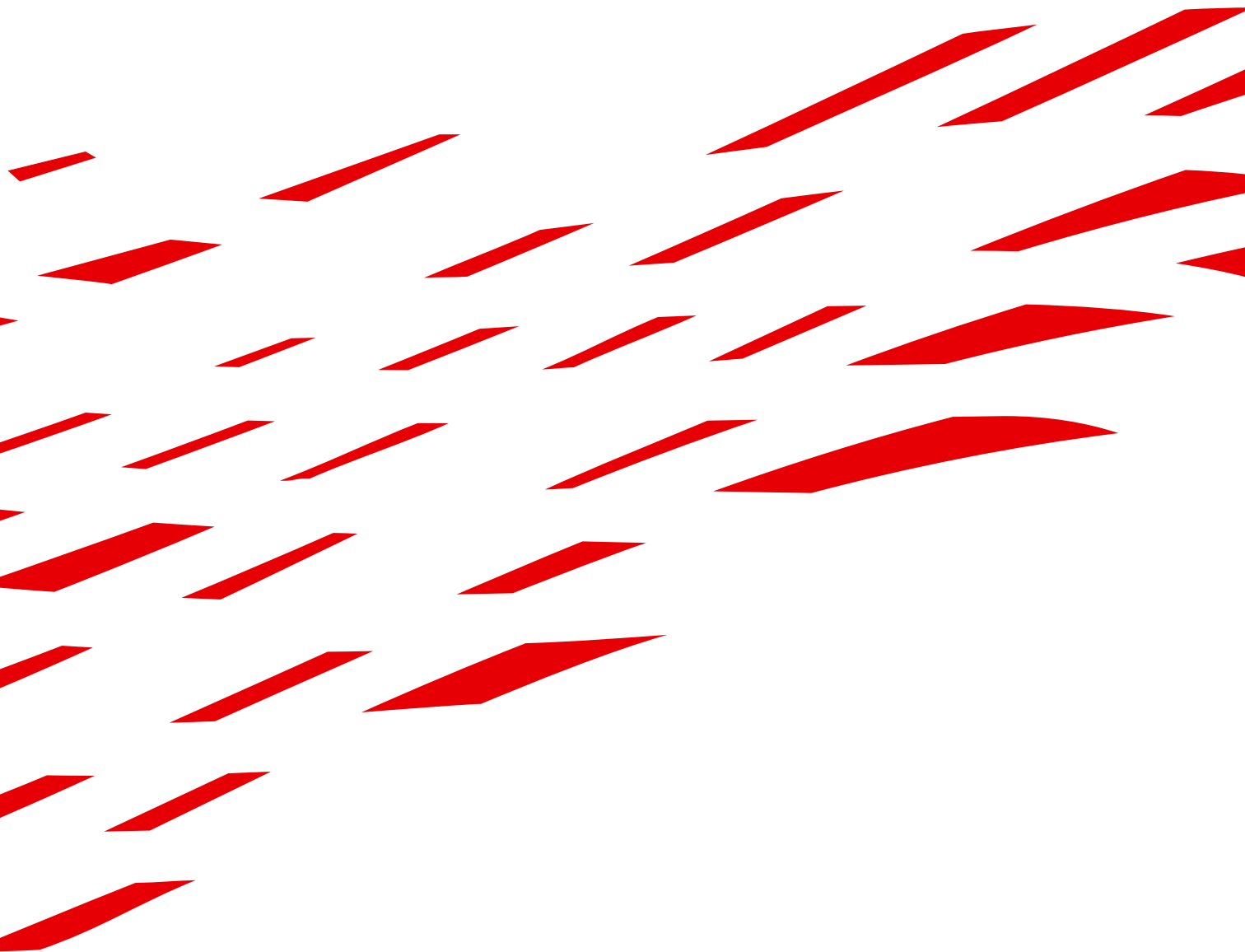
Several years ago, we embarked upon a challenging adventure: to bring together scientists from various disciplines to work on tissue engineering. This movement gathered momentum in 2004 with the launch of the Dutch Programme for Tissue Engineering, or DPTE. Now, three years into the programme, we can already say that our vision was right. Even in such a relatively short time, the different groups within DPTE have produced tangible results. Furthermore, it is clear that the synergy between the various DPTE groups has only just begun. As renowned stem cell researcher professor Christine Mummery says in this publication: 'When we started, the fields of cell biology and matrix engineering within DPTE were quite far apart. Now that we are half-way into DPTE, we are beginning to put the right questions to the tissue engineers. In the second half of the programme, I expect a lot of cross-fertilisation'. As one of those 'matrix engineers', working at the other end of the puzzle, I can only reaffirm the truth of that statement.

This publication shows where we stand after three years of research. It is intended as a journalistic impression, not a comprehensive report on all DPTE projects, and is aimed at the interested reader with some knowledge of the field. The introduction to each chapter and the quotes within the text should however give everyone a quick update on the progress we have made in the DPTE programme.

I hope you enjoy reading this publication. Meanwhile, we will continue with our cooperative research, to ensure the second half of the DPTE programme is even more productive.

Prof. dr. Jan Feijen

Chairman of the Board of the Dutch Programme for Tissue Engineering



1 *DPTE* Gathering the fruits of exploration and collaboration

The Dutch Programme for Tissue Engineering, or DPTE, was launched in 2004. By 2008, halfway through the six-year term of the programme, various groups have already produced promising results, in terms both of scientific publications and of steps towards clinical applications and valorisation. Collaboration within DPTE has already had an important impact on the generation of additional funds. The programme has now entered a new phase, in which collaboration between various groups is set to become even more important. Knowledge about the various components of living tissue is being combined, creating new prospects for large groups of patients.

As people grow older, their tissues and organs suffer from what the Bard called ‘the slings and arrows of outrageous fortune’. Trauma irreversibly damages tissues that have limited capacity for repair. At the cellular level, the accumulated damage to DNA leads to a deterioration in the functioning of cells or even cell death. Systemic diseases like atherosclerosis affect the vitality of many organs and tissues. Sometimes, surgical interventions are necessary. Though they may heal, they also leave defects that need repairing. And then there are the defects some children are born with, like heart valve deformations. Medical science can currently only partly solve these problems. A more creative approach is needed to help the body repair itself. An approach like tissue engineering.

The collaboration that started in DPTE has generated new initiatives in the fields of tissue engineering and regenerative medicine

Tissue engineering holds promise for millions of people now and in the near future, which of course means that it will also have a substantial economic impact. A rapidly growing number of people suffer from osteoarthritis, heart failure or bone diseases like osteoporosis. Many people have severe skin lesions that fail to heal with conventional methods or leave disfiguring scars.

All these patients, and the many more that are expected in an ageing population, are in urgent need of better treatments. Tissue engineering

might hold the key to the necessary clinical improvements, enhancing the quality of life of many people. In the future, even the patients now needing dialysis for kidney failure, the people who die of liver failure because of the lack of donor organs and even the many people suffering from neurological diseases like Parkinson’s disease, Alzheimer’s disease and stroke, may be cured by methods created in tissue engineering laboratories. The Dutch Programme for Tissue Engineering DPTE is laying the groundwork for these fascinating future developments.

FRAME 1

Collaboration

Tissue engineering is a fascinating field. It requires basic and practical knowledge about all the components of living tissues: cells, blood vessels, growth factors and other signalling molecules and the material around the cells (the extracellular matrix). To ‘engineer’ tissue grafts in laboratory, or to stimulate the repair of tissues in the body, it may not always be necessary to introduce all of these components. Sometimes, it might even be enough to introduce an optimal mixture of growth factors in the right dosage to help the body repair itself. But to accomplish this, a fair amount of knowledge is needed about all the pieces of the puzzle, as well as a research infrastructure to answer the more basic questions that arise on the path towards applications.

No single research group can study all aspects of living tissues and all the technical challenges of tissue engineering. That is why the DPTE programme’s main objective is to bring together biomedical scientists, engineers, clinicians and pioneering companies. Together, they work on scientific and technological solutions for practical applications of tissue engineering. Now, three years into the programme, most participants feel that a lot has been achieved, not only within the various groups, but also in the collaboration between groups. Scientists from fields as different as polymer chemistry and stem cell research have come together to work on important scientific

questions. They aim to standardize procedures in stem cell research, working towards safe clinical applications. In the field of scaffolds, collaboration has accelerated the development of optimal carrier materials for cells, often with a mixture of biological and synthetic components. Knowledge about growth factors is leading to the development of intelligent scaffold materials which release the right signalling molecules at the right rate to optimize cell growth and differentiation.

Another tangible impact of collaboration is the acquisition of additional funding from various other sources, often aimed at clinical and economic applications. In the last chapter, we will elaborate on programmes like SmartMix, Dutch FoRM and others, where the collaboration that started in DPTE has generated new initiatives for the fields of tissue engineering and regenerative medicine.

About this publication

In this publication, we will show some of the results and plans to emerge after three years of DPTE research. As you can see, much has been achieved and a lot of challenging projects are underway. Each chapter is devoted to a particular field of interest. First, the basic three platform technologies are discussed: stem cells, scaffolds and bioreactors. Then, two different fields of application are presented: cardiovascular tissue engineering and the fields of bone and cartilage tissue. We thus present highlights from both basic research and application-oriented research development. This is by no means a comprehensive presentation on all DPTE projects. Readers interested in some subjects, especially bone and cartilage research, are referred to several chapters, as both scaffold research and application-directed research deal with these subjects. We hope you enjoy reading this publication and will continue to follow the accomplishments of the DPTE in the scientific literature and at www.dpte.nl.

FRAME 1

DPTE: AN OVERVIEW

The DPTE is a consortium of research groups involving most Dutch universities and university medical centres, several research institutes, ZonMw (the Netherlands Organisation for Health Research and Development), STW (the Dutch Technology Foundation) and pioneering companies.

In the DPTE programme the multi-disciplinary field of tissue engineering is structured around three key platform technologies: stem cells, scaffolds and bioreactors.

Stem cells are cells with the potential to develop into several kinds of tissue cells. A material carrier (scaffold) seeded with stem cells or cells derived from stem cells could be used for several applications. Knowledge of stem cells is also essential in applications without cells, where the body's own repair cells have to be mobilised with growth factors and/or intelligent scaffolds. A lot of progress has been made in this field in recent years, but more research is needed. Specifically, the DPTE stem cell projects aim to:

- identify and isolate various stem cell populations;
- find the best way to stimulate their proliferation;
- induce differentiation towards a functional phenotype;
- obtain more insight into the interplay of growth factors, adhesion molecules and other factors in the growth and differentiation of stem cells and other cells.

Recently, DPTE researchers decided to standardize their operating procedures and develop new ways to characterize various cell types – a huge project with major implications both for scientific progress and for the development of future clinical applications.

The search is on for the optimal biocompatible and biodegradable **scaffolds**. Like the natural substances in tissue (the extracellular matrix) they provide different cell types with structural support and give the growing tissue its mechanical properties. In the course of time, the scaffold is replaced by the body's own extracellular matrix. Within the DPTE, both biological and synthetic degradable matrices are researched for different tissues, especially bone, cartilage, skin, heart muscle and blood vessels.

The bioreactor is where it all comes together. Researchers are seeking the optimal conditions for stimulating the proliferation and differentiation of tissue in a bioreactor. By modifying the physical, biochemical and mechanical environment, they eventually hope to perfect bioreactors for the generation of tissue grafts and eventually for the support of various vital organs like the liver, pancreas and kidneys. Bioreactors are also used to study the growth of tissues, to acquire the basic knowledge needed in regenerative medicine.

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Proefpersoon

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2 The promises and challenges of stem cells

Stem cell research is a major theme of DPTE. Some groups are addressing fundamental questions, while others are working towards clinical applications for building viable tissues. DPTE has been successful in bringing these groups together. Recently, stem cell researchers within the DPTE programme decided to work together on the standardisation of procedures and better ways of characterising different types of mesenchymal stem cells. Another major issue targeted in the programme is the interaction between cells and their environment.

No one doubts that stem cells hold huge promise for the future of medicine. Their ability to help regenerate damaged tissues and organs could enable us to treat a wide variety of medical conditions, especially diseases of old age like heart failure and arthritis (degeneration of the cartilage in joints). Stem cells and tissue engineering also offer hope of an alternative for donor organs. The shortage of donor organs for transplantation is a major problem throughout the world. At a time when all Western societies have an ageing population and growing numbers of patients with these ailments, it is hardly surprising that the promise of stem cells has caused something of a hype, both in the media and in scientific circles.

Adult stem cells have been called the universal ‘repair kit’ of our bodies.

Enthusiastic doctors have tried to cure patients with heart failure, for example, as well as other diseases, simply by injecting them with this 21st century panacea. Clever entrepreneurs are setting up services to capture stem cells from the umbilical cord of newborn babies and freeze them, in case they are needed for future medical treatments. Pay now, profit later (maybe). But the harsh reality is that no one, except some patients with blood diseases, has as yet been cured by stem cells. There have been some clinical trials in the fields of cardiology and neurology, but no major breakthroughs have been achieved to date. Promising as they are, the stem cells in our bodies have proven to be more elusive than most people expected. So there is more work to be done by scientists all over the world. In the Netherlands, much stem cell research is done under the umbrella of DPTE. In this chapter, we first discuss the principle sources of stem cells, before turning to a number of major issues in DPTE stem cell research.

Sources and types

There are several sources of stem cells, including embryos, the umbilical cord, blood and veins of newborns, and adult tissues. Embryonic stem cells are of vital importance for scientific research. Two DPTE groups studying these cells are for instance using them to elucidate how stem cells differentiate and what signalling pathways are involved. This information is crucial to our understanding of the developmental biology of stem cells, which is needed to control their behaviour in tissue engineering applications. Embryonic stem cell expert Prof. Christine Mummery (University of Leiden): ‘We regularly organise workshops to share our experience in culturing embryonic stem cells with other researchers who want to do their own embryonic stem cell research. The culture of these cells is notoriously difficult.’

Research on applications for human embryonic stem cells has serious ethical implications; in many countries, including the United States and Germany, the use of embryonic stem cells has been restricted because some people believe that every embryo with the potential to grow into a human being should be protected. Others claim it is unethical to abstain from research on embryonic stem cells, since it holds the promise of a cure for many patients suffering from debilitating diseases. In the Netherlands, derivation and use of stem cells from human embryos left over from in vitro fertilization for scientific research is permitted, but is not part of DPTE research. The creation of embryos specifically for research purposes is prohibited. Another source of primitive cells is the amniotic fluid which contains cells shed from the foetus. These can be collected in routine diagnostic procedures.

Umbilical cord blood is the most readily available source of stem cells. The umbilical cord and the placenta are usually disposed of after the delivery of the baby. The foetal blood in them can be collected and the stem cells harvested and stored. Most cord blood stem cells are used for haematological stem cell transplantations to cure diseases like leukaemia, but some are used in research. A subset of these cells, called mesenchymal stem

cells (MSC), have the capacity to differentiate into other cell types including fat, cartilage and bone. They are thus termed ‘multi (many) potent’. Other sources of similar stem cells include adult bone marrow and body fat. In fact many tissues carry an MSC population. These cells are designed to rejuvenate and repair the tissue by producing new cells. Though rare, they can be found. Recent research has suggested that some of these cells can be ‘reprogrammed’ to differentiate into tissues other than the tissue they originate from. So potentially, stem cells from fat tissue can be used to make bone or cartilage cells, but perhaps also liver cells. The progenitor cells already present in a tissue, like cardiac progenitor cells in the heart and stem cells from the skin, can also be used, however. Using adult stem cells could provide a perfect way of treating patients: stem cells derived from their own tissues could be grown in the laboratory and used to repair tissues damaged by disease, trauma or old age. The cells would be immunologically inactive, for they would be identical to the cells in the patient’s own body. Adult stem cells have been called the universal ‘repair kit’ of our bodies. And since no embryonic materials would be used, there would be no ethical issues other than the normal considerations in clinical research.

FRAME 2

Not only do the sources of the stem cells studied in DPTE differ, so does the type of stem cell. Most groups within the DPTE programme are working with mesenchymal stem cells. These cells are closest to or are already in clinical application. Other DPTE groups are working on embryonic stem cells and endothelial precursor cells.

Safety and monitoring

Not all stem cells are appropriate for clinical applications and some major challenges will have to be met before stem cell treatment in combination with engineered scaffolds complies with the essential safety standards. One of the risks is that stem cells may change into cancer cells. Optimal growth conditions and rigorous

Summary There are many different types of stem cells, depending on their source (embryonic, fetal, newborn, adult; different tissues like blood, fat, bone or skin; different stages of development). Some stem cells can differentiate into many different tissues, others are already ‘dedicated’ to a more specific type of tissue.

FRAME 2 TISSUE STEM CELLS: STEPS TOWARDS APPLICATION

Much research has to be done before patients can be treated with their own tissue stem cells. First of all the cells have to be found, characterised and purified. This requires detailed knowledge of their characteristics. The next step would be to expand them in a laboratory. Research focuses on optimal growth conditions and the signalling pathways involved in growth and differentiation. Then, cell differentiation has to be directed correctly and the cells have to be introduced into the body in such a way that they home to the tissues and organs where they are needed. All of these steps have to be defined with enough precision to reproduce them under GMP (Good Manufacturing Practice) conditions. Only then will it be possible to safely start the first clinical trials using these procedures.

FRAME 3 PROMISING NEW MOLECULE FOUND

Recent research within DPTE has led to an exciting discovery: a small molecule with immunosuppressive activity. The molecule is secreted when stem cells are cultured together with specific immune cells (dendritic cells). This molecule may be used to overcome the immunological obstacles to applications using stem cells and ‘engineered’ tissues. It may also be useful in transplantation medicine and in the treatment of some immunological diseases.

testing procedures are being developed to assess and reduce this threat.

When using stem cells from sources other than the patient himself, one has to deal with the problem of graft rejection. 'Foreign' cells will be recognised by the immune system of the patient and cause an immune response. So just as in an organ transplant, the patient would have to be 'matched' with donor cells and treated with medication to suppress the immune response. This has many long term side-effects.

FRAME 3

The cells currently grown in research laboratories have another disadvantage: they are mostly grown in media containing products derived from animals. Because of infection risk and standardisation problems, the use of animal products in clinical applications is highly regulated. Many products are not allowed in the production of cells that will be used in humans. One of the challenges for stem cell therapy is to develop standardised media that do not contain animal products.

Monitoring the fate of the implanted stem cells and scaffold breakdown products is essential to meet the demands of European regulations, which require that the fate of anything introduced into the body be known before approval for clinical use is given, as for drugs. Earlier research has shown that it is not easy to distinguish implanted cells from the normal host cell populations. Special procedures are being developed to make certain that the right cells are being monitored. Following the fate of implanted cells is also very important in a research setting, to understand why many grafts are not sustained, causing loss of functional improvement over time.

Characterisation high on agenda

To use stem cells in clinical applications, one must of course know exactly what type of cell one is dealing with. It is not enough simply to know the source of the cells; one must also be confident that the cells will develop in the required direction. If

one cannot predict and control the direction of differentiation, it can be difficult or even dangerous to use stem cells in patients. No one wants to introduce bone tissue into the heart, or use muscle cells to heal a broken bone.

Characterisation of different subtypes of stem cells is therefore high on the agenda of DPTE scientists. Different approaches can be taken to characterise cells; a combination of these tests will probably eventually become the gold standard.

For clinical applications, living cells sorted from mixed populations of different cell types would be needed. This can be done by identifying protein markers on the surface of the cell and using them to select cells with specific antibodies, as is already happening in the analysis of haematological stem cells that derive from the bone marrow. Specific markers for other types of stem cells are now being studied.

FRAME 4

The need for standardisation

When the DPTE programme started in 2004, scientists were not yet fully aware of the level of complexity involved. Now, four years into the programme, they realise that more basic knowledge is needed to make stem cell therapy work in a clinical setting. Professor Carl Figdor (Radboud University, Nijmegen) is on the board of the DPTE programme and a leading expert on immunotherapy and the use of stem cells. He explains how, as a scientist, he sometimes has to disappoint people who are eager to apply the new stem cell biology in medicine. 'There is an enormous drive towards practical applications. Expectations are so high that some people throw caution to the wind and start clinical trials before there is reasonable scientific evidence that the therapy will work and has no harmful side-effects. I think it is wiser to be honest and say that we cannot as yet fulfil the expectations in the clinical field. All we can do is respect the facts. And at the moment, the fact is that we need to know much more about the cells we are dealing with and the optimal procedures to harvest, characterise and culture them. That is where DPTE is very useful, because it provides a platform for the different

groups working with stem cells to decide on optimal procedures.’

One of the problems scientists have to tackle is the fact that, up to now, every laboratory has developed its own procedures. Furthermore, they use stem cells from different sources (cord blood, bone marrow, foetal cells from amniotic fluid, fat tissue). The results in one laboratory are not easily reproduced in another, making the debate on scientific results very difficult. Put simply: when a researcher talks about stem cells, he may be speaking about quite another type of cell than his colleague from another lab. And the advantages and disadvantages of different sources and lab procedures in terms of differentiation and application are largely unknown. This is a major disadvantage for scientific research and, even more importantly, for clinical applications, where every procedure has to be tested and documented. Standardisation is essential to overcome these problems, but it will be no easy task. It is only too human to assume that the way things are done in your own laboratory is the best way. So it is not easy if you have to change the way you work and adopt a procedure from elsewhere. But the cooperation within DPTE should help overcome these barriers and establish common ground among the Dutch scientific stem cell community. This will pave the way to the next step in research development - working towards clinical applications. According to Figdor and Mummery, the Netherlands has an excellent tradition of scientific cooperation – in addition to a healthy competitive spirit. Hopefully, over the next few years, this cooperation will provide more clarity in the field of stem cell research.

FRAME 5

Summary Since there are many types of stem cells and even more procedures to handle them, standardisation of procedures and clear consensus about terms and names are a crucial next step. Standardisation of procedures is very important in science, and also in developing materials for clinical use. DPTE has created a network of pioneering stem cell scientists, working on this highly needed clarification.

FRAME 4 HAEMATOLOGICAL STEM CELL THERAPY

Bone marrow transplants performed at specialist clinics are the oldest stem cell therapy, the first having been performed 40 years ago. The stem cells in bone marrow produce all types of blood cells. In patients with leukaemia and some other haematological diseases, a transplant with these haematological stem cells can be used to replenish the bone marrow after intensive treatment. We know a great deal about the surface characteristics of haematological stem cells, and they can be separated into different subsets. Cells with unwanted characteristics, like certain types of immune cells, can be eliminated from the graft.

FRAME 5 PUTTING EVERYTHING TOGETHER

All the tissues studied in DPTE consist of cells and an extracellular matrix. To replace such tissue, knowledge is needed both about components and about their interaction. Scientists growing cells in the laboratory often need matrix components keep the cells ‘in shape’. In developing safe ‘scaffolds’ for tissue engineering, one cannot ignore cell biology. One of the major accomplishments of DPTE is that cooperation has been established between cell biologists, tissue engineers working on artificial matrix components and scientists developing bioreactors. Basic research has provided a great deal of information about the way stem cells interact with their biochemical environment, through specific receptors on the cell surface. Physical properties of the surrounding extracellular matrix such as elasticity and hardness also influence cell behaviour. In years to come, the cooperation between tissue engineers and biologists in DPTE will provide scientists and clinicians with new ways to control cell behaviour through matrix properties. Mummery: ‘When we started, the fields of cell biology and matrix engineering within DPTE were quite far apart. Now that we are half-way into DPTE, we are beginning to put the right questions to the tissue engineers. In the second half of the programme, I expect a lot of cross-fertilisation’.

IMMUNOMODULATION AND REPAIR One successful research group working towards clinical applications of stem cells is that headed by Prof. Wim Fibbe of the Department of Immunohaematology and Blood Transfusion at Leiden University Medical Centre (LUMC). He and his coworkers have years of experience in developing Good Manufacturing Practice (GMP) protocols, an essential step towards clinical application. They use stem cells derived from bone marrow for two different (but possibly related) purposes: to influence the immune system and to modulate repair processes.

Mesenchymal stem cells sometimes help to suppress the immune system. The researchers are using this principle to address the two major clinical problems that occur in patients undergoing haematological stem cell treatment ('bone marrow transplant'): graft rejection and graft versus host disease. Sometimes, after haematological stem cell transplantation, the graft is destroyed by the remaining immune system of the patient. In a clinical experiment, mesenchymal stem cells from the donor are expanded in the laboratory under GMP conditions, five weeks prior to the actual transplantation. They are then added to the mixture of cells given to the patient. So far, in this international project being conducted with the University of Pavia, Italy, 14 children have undergone this treatment. Up to now, no rejection has been observed. The graft itself also contains immune cells. They can recognise the body cells from the

host as foreign and start to attack them, causing so-called graft versus host disease. In severe cases, this can be a life-threatening condition. Sometimes, graft versus host disease does not respond to any available treatment. In these cases, stem cells could modify the immune response. This is now being studied. Some diseases involving the immune system could also be treated using this approach. In a pilot study, the researchers are attempting to treat severe cases of autoimmune inflammation of the bowel (Crohn's disease).



Repair

Another project is studying the effects of mesenchymal stem cells derived from bone marrow on repair processes in the heart after myocardial infarction. Earlier animal studies have already shown that these stem cells can modify the formation of scar tissue. This may improve functional recovery of the heart muscle. The exact

mechanism by which the stem cells may modify the repair of the heart muscle is as yet unknown, though it has been suggested that the treatment works by reducing the inflammation in the infarcted area. But the cells may also influence the process of scar formation and the growth of new blood vessels.


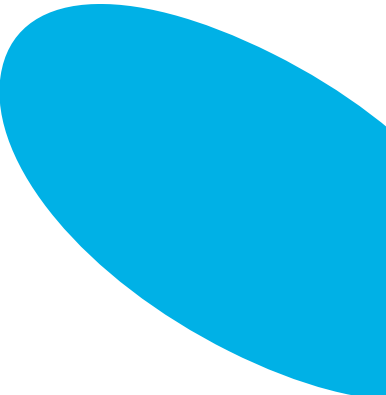
'We are the only group so far to use mesenchymal stem cells in clinical experiments', says Fibbe. 'It requires a huge effort to get the research from the lab into the clinic. It takes at least a year and a half to prepare experiments like these, writing standard operating procedures, validating all growth media and practising procedures to meet GMP standards. You have to check and double check every step and record everything you do, so if anything goes wrong you can evaluate the whole procedure. It is expensive, it is complicated, so I think production under GMP conditions will remain highly centralised.'





3 Engineering the matrix

Extracellular matrix is the natural material in which living cells grow and function. To engineer a tissue graft, a synthetic matrix, or 'scaffold', must be used to provide a safe environment for cells to grow in. In the first years of the DPTE programme, a lot of work has been done on the development of high-quality biomaterials that can be used as scaffolds. Now, the time has come to study the interaction of these materials with the cells that will have to grow in them to create a living tissue graft.



'The ideal biomaterial to use as a scaffold will provide stem cells or cells from the body with an environment that will optimise their chances of survival and guide their differentiation into the required tissue cells', says Prof. Jan Feijen, Professor of Biomaterials and Polymer Chemistry at Twente University (Enschede) and chairman of DPTE. 'To accomplish this, the biochemical and microscopic properties of the material should be 'cell friendly' and its mechanical properties should match those of the surrounding tissue, to allow transmission of mechanical stimuli to the cells. It should be possible to use it to deliver growth factors to developing cells. Our ideal biomaterial would contain no toxic or infectious agents, even if it were of biological origin. And it would be biodegradable, being gradually replaced by the natural extracellular matrix of the body produced by cells. The degradation products must of course also be non-toxic and easily removed by the body. Nor should the biomaterial cause adverse tissue reactions. For most tissues, the possibilities of vascularisation must be considered, both during the production of the graft in the laboratory and after transplantation.'

'Bone from a syringe': orthopaedic surgery's future tool?

Feijen's wish list shows the many challenges researchers trying to develop scaffolds have to face. A material may look perfect from a mechanical point of view, but fails to meet the safety criteria, or for some reason may not be as hospitable to cells as it first appeared. Luckily, several research laboratories in the Netherlands have a toolkit of technical tricks that enables them to develop many different materials, engineering their properties until they meet the demands of tissue engineering. One good example of the technical challenges involved in the development of suitable materials is the incorporation of growth factors into different

kinds of scaffold. To stimulate cell growth and differentiation, the cells seeded on the scaffold will need the right concentrations of several growth factors over time. Simply injecting these factors will not work, as they are easily washed away in a watery solution. Engineers have developed techniques to incorporate growth factors into the scaffold material in such a way that they are gradually released.

Thanks to the DPTE network, a lot of interaction has been established between engineers researching scaffolds and biologists studying cells.

Collaboration between the two is essential to make tissue engineering a clinical reality. Cross-fertilisation has also occurred between different research groups working on scaffolds. Each of them has its own expertise, and often the combination of different fields of expertise yields new solutions to complex problems. Research on scaffolds is often focused on a specific type of tissue. The results obtained so far in these different fields are briefly discussed below.

Bone

Bioceramics (calcium phosphate-like materials) are the materials of choice for bone replacement procedures. Their properties may not exactly match those of natural bone, but they do bind chemically to the surrounding bone, keeping the graft in place. They also provide a surface for bone ingrowth (osteoconduction). The challenge is to develop a bone substitute that can be used to bridge the gaps in the larger bones of the extremities caused by trauma or surgery. These will have to withstand strong forces, requiring a material that is both rigid and elastic.

At the moment, DPTE researchers working on bone have two goals: the development of 'bone from the syringe' (a ceramic for use as an injectable bone substitute) and the engineering of bioceramic scaffolds with the right three-dimensional structure and the possibility to include small bioactive particles (i.e. growth factors) within the scaffold. Different approaches are being pursued to achieve the latter goal. In Nijmegen, a new Electrostatic Spray Deposition (ESD) apparatus has

been developed. It successfully deposits calcium phosphates (CaP) and biological agents (proteins such as growth factors) with a wide variety of tailored surface properties. The electrospinning technique (another kind of spraying) is used to create nanofibres with different properties. The bone-forming properties of polymers can be enhanced by coating them with a layer of bone-like apatite or CaP ceramic. In-vitro cell culture studies show considerable differences in cell responses towards the various coatings.

FRAME 6

Cartilage

Tissue engineers are pursuing several options to find an optimal scaffold for cartilage. In Utrecht, synthetic polymers that may be used as a cartilage scaffold have been developed. Researchers were able to create materials that are solid at body temperature, which is important to make porous structures. Furthermore, these materials can easily bind and release proteins, growth factors and other useful molecules. These can be used to influence the cells that are seeded in the scaffold and help them bind to their material surroundings.

Polymers that can form hydrogels are being studied in Utrecht and Enschede. These hydrogels readily absorb water, facilitating exchange of oxygen and nutrients with the surroundings. Chemical modification and ultraviolet light were used to successfully overcome the problem of rapid degradation of these hydrogels under body temperature conditions.

FRAME 7

Fibrinogen, a blood-clotting protein that can polymerise to fibrin networks, is also an interesting candidate for several scaffold applications, including cartilage and skin. In Amsterdam, methods have been devised for the purification of different forms of fibrinogen from

Summary In search of the best 'scaffold' biomaterial for bone and cartilage, several chemical and biological approaches are being pursued. The inclusion of growth factors could contribute to tissue growth within a scaffold.

FRAME 6 MICROSHERE

MAGIC The inclusion of microspheres containing synthetic or organic materials in calcium phosphate cement increases the porosity of the material. This has a great impact on new bone formation. Even without the use of growth factors, some of these particles made the cement capable of bridging critically sized defects.

In Eindhoven, several physicochemical approaches were used to create calcium carbonate materials that look exactly like the minerals in natural bone. Researchers succeeded in producing thin continuous coatings of calcium carbonate that had favourable characteristics in in vitro testing.

FRAME 7 'TWO-COMPONENT GLUE' HYDROGELS

Researchers in Enschede are also working on hydrogels with a special property: they form from two components, like some kinds of superglue. After injecting and mixing the two components together, the gel takes several minutes to set, allowing the surgeon time to mould the gel into the required shape. The physical properties of this hydrogel can be influenced by visible light, yielding a gel with more rigidity. The degradation of the gel under biological conditions can also be influenced. This research has already led to a new patent application. The next step will be to introduce cells and biologically active components into this hydrogel scaffold.

FRAME 8 HEALING HERNIATED SPINAL DISCS THROUGH A SYRINGE

Collagen is the most prevalent protein in natural cartilage. In Amsterdam, progress has been made towards the development of injectable scaffolds that could be used to mend defective (herniated) intervertebral discs, a common cause of debilitating back pain. Researchers have studied the behaviour of stem cells in hydrogels made from collagen type I and II and alginate (a natural product made from algae). One very interesting development was the production of large quantities of recombinant human procollagen by biotech company Pharming BV. Several variants of collagen will be prepared from this procollagen. The next step is to seed these scaffolds with living stem cells (derived from adipose tissue) and study the behaviour of these cells using various state-of-the-art technologies.



human plasma. Small amounts of fibrinogen in its different forms have also been made in the laboratory using recombinant DNA technology. Unlike fibrinogen from organisms, the products of recombinant technology are very pure, allowing very specific definition of the molecules. The methodology has been developed to study various aspects of the material, using electron microscopy and other techniques. The effects of fibrinogen variants on cell growth will now be studied.

FRAME 8

Small blood vessels

The material properties needed for the engineering of small blood vessels are quite different from the applications discussed so far. The material has to be flexible, allowing for the characteristic pressure pulsations in the blood circulation. It also has to be strong enough to withstand the pressures involved from the moment it is implanted into the body. And of course, it must provide a good scaffold for the growth of endothelial cells and smooth muscle cells lining a blood vessel. Another approach is to use stem cells in combination with the scaffold, with the stem cells differentiating into the required blood vessel cells. Researchers in Enschede have been successful in the preparation of polymers that are promising candidates for application in blood vessels and myocardium. They used gamma radiation to make these polymers cross-link, rendering the materials flexible and elastic. Different radiation doses can be used to fine-tune material characteristics like degradation rate. According to tests in cell cultures, these polymers were non-cytotoxic and biodegradable. The next step is to develop and test a living blood vessel under natural conditions. To this end, a pulsatile bioreactor system is being set up, using mesenchymal stem cells to populate the biomaterial (see page 34).

4 Controlling the parameters for tissue growth

Bioreactors provide an optimal environment for the growth of tissues. Research in this field aims to develop the technology to grow tissue grafts that can be used in clinical applications. Bioreactors are also powerful tools for studying tissue growth. The knowledge gained in this research will also be applied in other approaches to regenerative medicine. The patient's own body may just prove to be the best bioreactor for growing bone, cartilage and blood vessels.

Bioreactors are the alchemist's oven of the 21st century. Here, the miracle of tissue engineering actually takes place. From cells and carrier materials (scaffolds), a whole new piece of tissue develops, under carefully controlled conditions. Bioreactor technology is well suited for automation, which may in the future help to lower production costs. The main difference between normal cell culture and the growth of tissue in a bioreactor is the extent of control. Normally, cells are grown in flasks containing a medium. Fresh medium is added every other day. This works fine for many cell types, but for tissue engineering applications, it is important to be able to control the biochemical and biomechanical conditions in real time. Many variables have to be monitored and adapted to optimise cell growth and differentiation.

A living heart valve grows with the young patient's heart.

Mechanical influences like pressure are sometimes needed to give the tissue the right properties. So for each application, a special type of bioreactor has to be designed and tested. Within the DPTE programme, the main applications being studied are the development of cardiovascular tissues (heart valves and small vessels) and cartilage. In a related project, technology is being developed to monitor growing tissues (bone and cartilage) in the bioreactor. The rest of this chapter discusses these three projects.

Living heart valves

Every year in Europe, an estimated 6000 children are born with severe congenital heart valve defects, especially in the right half of the heart, affecting the pulmonary circulation. Conventional heart valve surgery has little to offer these newborn babies, because their heart will rapidly outgrow any artificial heart valve prostheses. What is needed is a

heart valve that will not only function after implantation, but also grow with the heart and adapt to the demands of the body. Researchers at Eindhoven University of Technology aim to develop such valves, grown in a bioreactor from the patient's own cells. And they have come a long way already.

The challenge is enormous. Every day, a heart valve has to open and close more than 100,000 times without a moment's rest. It has to withstand a pulsating pressure, operating smoothly without interfering with the blood flow. And it has to continue to function and adapt for many decades. Until now, only Mother Nature has been able to accomplish all this. So the first step in developing a bioreactor for the growth of living artificial heart valves has been to mimic the biomechanical conditions in a human heart.

Living cells (myofibroblasts on the inside and endothelial cells on the surface) are seeded on a biodegradable polymer scaffold and grown under conditions of pulsatile mechanical stimulation. In the course of 3-4 weeks of cultivation within the bioreactor, much of the polymer is degraded and replaced by collagen fibres produced by the myofibroblasts. Thanks to the mechanical stimulation, the architecture of these fibres develops a close resemblance to the natural heart valve of a newborn baby. This also means that the artificial heart valve potentially has the strength and other biomechanical characteristics needed for functioning in a young human patient. Animal studies in sheep have shown that the heart valves remain functioning and adapt to the host for at least six months. Long-term animal studies will now have to be conducted to prove that these bioreactor-grown heart valves can last a lifetime. A lot of time and energy have been invested in developing the best bioreactor, allowing for optimal control and sterile, safe conditions throughout the weeks of tissue growth. Another important consideration is how best to mechanically stimulate the growing tissue. As in sport, the best training regime for the growing tissue has to be identified. Continuous pulsatile simulation like in the natural situation may not be the best way to train a growing

heart valve. In the beginning, it might be best to allow the cells some rest between training periods. The optimal biochemical conditions also need to be verified.

FRAME 9

FRAME 10

Adult heart valves

Each year, 300,000 adults worldwide need a new heart valve. Current solutions all have their disadvantages. Heart valves of biological origin (specially prepared heart valves from animals) are used in older people. But these valves often only last for 15 years. Mechanical heart valves, often used in younger patients require life-long anticoagulation therapy, with serious side effects. Reoperations are often necessary, made complicated by scar tissue formation. So if this bioreactor development of heart valves is successful, it may also give adult patients a lasting solution to their problems. From a commercial point of view, these 300,000 heart valves represent an interesting market. A company has in fact been set up in connection with the DPTE project and a related SmartMix project to produce heart valves under GMP conditions and perform the (pre)clinical testing of the valves in collaboration with Zurich University Hospital and Utrecht University Medical Centre, once the long-term animal studies have been successfully completed.

Cartilage engineering

Tissue engineering of cartilage seems relatively easy (although it proves to be quite a challenge in practice). The tissue contains no blood vessels and only one type of cell, the chondrocyte. These cells depend on diffusion through the tissue matrix of oxygen and the essential nutrients. Under normal body conditions, this means that chondrocytes are accustomed to relatively low oxygen levels. A bioreactor mimicking physiological conditions will need to provide such a low-oxygen environment, allowing for the monitoring and control of oxygen levels and other variables. In Rotterdam, a commercially available bioreactor

Summary A bioreactor provides a good environment for tissues to grow in. Circumstances can be monitored and regulated, optimising the development of a healthy, growing tissue. Living growing heart valves for newborn patients (or adults) are an exciting example. The generation of living cartilage is another.

FRAME 9 A CLOSE WATCH ON GROWING VALVES

In tissue engineering, it is essential to monitor not only the biochemical and biomechanical input variables, but also to know if the material under development has the required mechanical characteristics. A major breakthrough was therefore the development of a new method to non-invasively assess the deformation of heart valve leaflets. This has led to a patent application. The researchers will implement a feedback control bioreactor, using this information about the biomechanical status of the growing heart valve to optimise its further development.

FRAME 10 HEART VALVE DELIVERED ON TIME

Several alternatives are possible in the future. Collaboration with Zurich University Hospital may yield a new approach, using cells from the amniotic fluid. This would make it possible to start growing the heart valve from the moment diagnosis of congenital heart disease is possible, at 20 weeks' gestation. By the time the baby is born, the heart valve may already be on the shelf, ready for implantation.

was modified to culture cartilage. The setup became a tool for studying several metabolic processes involved in the formation of cartilage.

It has become clear that not only oxygen levels, but also acidity (pH) influence the chondrocytes' biological behaviour. The bioreactor allows researchers to manipulate these variables (oxygen pressure and pH) independently – in normal cell cultures, a low oxygen level automatically leads to a higher acid (lactate) content. Now, the optimal conditions in the medium for cartilage development can be studied. Glucose content may also be an interesting parameter to 'play' with in these experiments.

One major challenge is to keep the chondrocytes in shape. Without the right environment, the cells rapidly dedifferentiate and lose the ability to produce the right amounts of matrix proteins (collagen and proteoglycans). In a new approach, developed within the project, chondrocytes are attached to the surface of microbeads. The convex shape of the beads has turned out to be important in keeping the chondrocytes in their correct phenotype, producing the hyaline cartilage that is characteristic of articular joints.

To better study the behaviour of chondrocytes in the bioreactor, reporter gene constructs are introduced into the cells. These genes can be specifically attached to the genes that are important in cartilage protein production. If the cell starts to produce these proteins, the reporter gene is activated and the cell begins to emit fluorescent light. This gives researchers the opportunity to identify the moment that cells become active under various biochemical and biomechanical conditions.

Future developments

Mechanical stimulation is also important for cartilage formation under physiological conditions. In a joint, the compression of the cartilage whenever we move enhances the diffusion of molecules to the chondrocytes. It also influences their biological behaviour and the architecture of fibres in the matrix. So the next step in the development of an optimal bioreactor for the

generation of cartilage may be the introduction of mechanical stimulation.

At the same time, the research in the bioreactor may help us to understand these cells, offering the prospect of other approaches, like the injection of chondrocytes into a lesion. This could obviate the need for bioreactors in clinical applications. At the moment, it is impossible to predict which approach will become the future standard.

Continuous real-time monitoring

Tissue grafts grown in a bioreactor will eventually have to function within the body. The same is true of other applications in tissue engineering, like cell transplants, the implantation of intelligent scaffolds or the application of growth factors to regenerate tissues. To predict the effects and optimize these approaches, it is highly important to have enough knowledge on cell behaviour in a three-dimensional (scaffold) environment. The bioreactor could generate such knowledge if cells, tissues and scaffold materials can be studied at a microscopic and molecular level.

In Enschede, a system for studying these aspects is being set up, using a new technology called confocal Raman microscopy. Confocal microscopy allows the three-dimensional properties of a living tissue to be studied by looking 'through a pinhole'.

By illuminating only a small part of the tissue and moving that illuminated spot through the tissue, the architecture on a microscopic level is revealed. Raman spectroscopy uses the way different molecules influence light to follow the fate of various relevant biomolecules within the tissue. Together, these techniques yield a highly informative 'movie' of the developing tissue, without interfering with its function (and of course, without having to destroy the tissue, as in conventional microscopy). This technology is now being developed and tested, with special emphasis on the information needed to grow cartilage and bone. Continuous real-time monitoring has the potential to become one of the major 'tools of the trade' for tissue engineers.

THE CHALLENGES OF ENGINEERING SMALL BLOOD VESSELS

In cardiovascular surgery, there is a great need for blood vessels to bypass obstructed arteries, in the coronary vascular system and elsewhere in the body. The replacement of big blood vessels like the abdominal artery with Teflon grafts is already a routine procedure. But for smaller vessels, with a diameter less than six millimetres, no usable artificial prostheses have been found as yet.

Smaller blood vessels made of artificial materials tend to become obstructed by blood clots. In bypass surgery, blood vessels from patients themselves need to be used. This approach obviously has its limitations. Only a few blood vessels can be used, and these also tend to be vulnerable to obstruction.

Researchers in Eindhoven have developed a bioreactor for growing smaller blood vessels. The approach is similar to the tissue engineering of heart valves. The same cell types are used and the growing tissue grafts are also subjected to mechanical stimulation. The difference, however, is that a blood vessel needs to withstand continuous pressure, combined with pulsatile pressure peaks. It will have to remain open and not cause blood clots in the body, while at the same time being strong enough not to widen too much, developing into an aneurysm.

Hope for kidney patients

This research is now moving into a new phase. Preclinical studies are being conducted with Maastricht University Medical Centre. The plan is to test the artificially grown blood vessels in animal studies first, and if this is successful, to develop a bioreactor-grown blood vessel as

a so-called A-V shunt for kidney patients who need dialysis. Currently, polymer-based artificial vessels are used as A-V shunts, but they have proved vulnerable to infection and frequently only last about a year to eighteen months. Researchers hope to improve on this with a tissue-engineered blood vessel.

Plans for further research

In the future, researchers plan to study other scaffold materials, possibly mimicking the layered structure of the normal blood vessel. The knowledge gained in bioreactor research may also lead to the development of cell-free implants from intelligent scaffolds that are able to attract the right cells from the bloodstream. In this scenario, the body becomes the bioreactor. One advantage of this approach would be that surgeons would have the right vessels ready at the moment they were needed, without the delay involved in growing an artificial cell-populated blood vessel in a bioreactor. It would also almost eliminate the risk of infectious contamination. But of course, a lot of specific knowledge about scaffolds and cells will be needed before this approach can be tested in humans.





5 Moving towards better mobility

The work on bone and cartilage within the DPTE programme has been very successful. Some of the findings are already close to experimental clinical application. More research is being done to meet the challenge of providing millions of people with better functioning joints or repairing their spine and bones. The research focuses on both scaffolds and cells. There is a strong emphasis on the application of growth factors and smart scaffolds to stimulate regeneration.

In an ageing population, research on the regeneration of bone and cartilage is of the utmost importance. More than half a million people in the Netherlands suffer from osteoarthritis in the hip or knees. In this condition, the cartilage of the joint has degenerated, causing pain and, eventually, impaired mobility. The number of people suffering from this typical disease of the third age is growing rapidly.

In young people, cartilage can be damaged by trauma due to traffic accidents or in sports, as everyone who watches soccer knows. Since this tissue does not spontaneously regenerate, surgical interventions are the only way to cure a defect. There is a growing need for surgery of the spine, to treat problems with the vertebrae (bone) and the intervertebral discs (cartilage). Current standard surgery is invasive and recovery is often painful and protracted. New approaches are needed.

Bone defects can be caused by trauma, or can be an inevitable effect of bone surgery. Bone is one of the most frequently used tissues for transplantation. The gold standard here is still the use of bone grafts from other parts of the patient's body (autologous bone grafts). But there are several major disadvantages to this approach, especially in elderly people, if only because their autologous bone may not be of the required standard. The development of alternatives to autologous bone is a very relevant issue in bone reconstructive surgery.

*Artificial cartilage
will heal patients
with osteoarthritis or
sports injuries.*

The work on bone and cartilage within the DPTE programme has already yielded significant scientific results. They are on schedule, but a lot of work still has to be done before the results from laboratory research can routinely be applied in a clinical setting.

This chapter discusses the accomplishments achieved within the DPTE programme. First, we present the results of cartilage research, followed by the results of bone research. We conclude with a brief discussion of applications in clinical practice.

Cartilage

Cartilage lines the joints of our body, ensuring smooth and painless movement of the joint. It is also present in airways (nose, trachea) and the external part of the ear. There are different types of cartilage (elastic, hyaline and fibrocartilage), each suited best for the biomechanical conditions in which it must function. A cartilage lesion will result in pain and functional impairment. Damaged cartilage is unable to repair itself. There are not many cells in cartilage anyway, and they cannot move through the tissue. Furthermore, it contains no blood vessels to bring in repair cells.

The only way to repair cartilage surgically is by using pieces of cartilage from another site in the body. Obviously, this is not an ideal situation. To really heal a cartilage defect, regeneration has to be stimulated, either by importing repair cells to the lesion site or by stimulating the regenerative capacity of the chondrocytes within the remaining tissue. Research focuses on the isolation and cultivation of different cell types and on optimising their local conditions for repair.

Bone marrow-derived cells and chondrocytes from the ears and joints are being studied as part of the DPTE programme. Both human cells and murine cells are being used to address various questions on the cell biology of different cell types.

Many different approaches to the formation of cartilage (chondrogenesis) from different cell types are being studied simultaneously in different projects. To prompt cells to produce new cartilage, scientists are applying soluble factors, growing different types of cells together, introducing new

genes into the cell, manipulating the mechanical properties of the materials cells grow on and, last but not least, using different scaffolds to optimise conditions for chondrocytes. Each of these approaches has yielded new insights.

The research on different cell types has shown that the flexibility of cells must be taken into consideration. This is especially true of bone marrow-derived stem cells, which have the potential to differentiate into different cell types. Special measures will be needed to keep these stem cells on track. Even chondrocytes themselves can lose their specific properties in culture.

FRAME 11

Cartilage scaffolds

Last but not least, the development of suitable scaffolds for the growth of cartilage in the laboratory is being investigated (see also page 19). This has proven to be quite a challenge. Some synthetic scaffolds do not degrade fast enough and are rejected by the body. A polymer with better resorption characteristics (lactide-caprolactone) showed good biocompatibility. But for applications in articular joints, the surface of these scaffolds is not smooth enough to allow frictionless movement in the joint. Research now focuses on combinations of polymer scaffolds and natural materials like collagen. The mechanical properties of the scaffolds will be mechanically tested with a biomechanical compression tester being developed under the DPTE programme. And finally, a major step is being investigated: what happens to chondrocytes and their precursors if they are cultured in different scaffold materials? (See page 24)

Bone

Research on the regeneration of bone and dental elements can be divided into more fundamental research, which attempts to elucidate the interaction between cells and their environment and work on clinical applications. In the former category, a lot of research focuses on the generation of functional bone grafts in the laboratory. This may yield some practical

Summary Cartilage does not regenerate spontaneously. Both the cells and the extracellular matrix of cartilage are being studied, providing new possibilities for patients with damage to this tissue. Basic science and clinical research are also working on solutions to repair major bone damage.

FRAME 11 PEACE WITHIN THE JOINT: 'JOINT HOMEOSTASIS'

One problem that has to be overcome is the fact that a cartilage lesion site seems to be a hostile environment for repair cells.

This can be seen on the outside of a damaged knee: the swelling, pain and redness indicate an inflammatory response by the body.

The formation of new cartilage is strongly inhibited by this micro-environment, so effective repair depends upon the ability to 'restore the peace' within the joint ('joint homeostasis'). DPTE research focuses on the identification of factors that break down newly-formed cartilage and anabolic factors that promote the formation of new cartilage tissue.

The next step is to find ways to inhibit breakdown and stimulate regeneration. This is done by introducing genes. The gene therapy approach may lead to direct clinical application, or at least increase our knowledge of how chondrogenesis is regulated in cartilage lesions.

applications in the future, but according to clinicians it is more likely that the knowledge gained in fundamental research will lead to other, more practical approaches in clinical practice (see below).

In the laboratory, bone-forming cells, or osteoblasts, can be grown from mesenchymal stem cells derived from the bone marrow. Clinical trials demonstrate that these stem cells can form bone tissue upon implantation. But the amount of bone formation achieved so far has not been enough to enable bone defects to be bridged. According to the researchers, this could be caused by limited graft survival due to insufficient nutrient supply. This emphasises the importance of vascularisation. Several strategies are being pursued to support the ingrowth of endothelial (precursor) cells and the formation of new blood vessels. The poor results from clinical studies can also be explained by the absence of sufficient bone-forming triggers after implantation of the graft.

The application of bone marrow-derived stem cells in itself is not without practical problems. Harvesting and expansion of these rare cells from the bone marrow under GMP conditions is time-consuming and raises the cost of the grafting procedure. And it has been found that stem cells lose some of their bone forming capacity if their numbers are expanded in cell cultures, mostly due to premature ageing of the cells. The role of DNA damage in this process is now being investigated. Different approaches are being studied to influence the behaviour of stem cells. More than 1800 different compounds have been screened to find the 'magic bullets' that might enhance the formation of bone in culture-expanded mesenchymal stem cells. A handful of promising candidates have been found. Interfering with the expression of certain genes with special RNA-molecules is another promising approach that might lead to practical applications.

The differentiation of stem cells into bone cells also depends on the biomechanical characteristics of the material they grow in. A certain stiffness is needed in the scaffold for bone formation. Researchers have developed tools to quantify scaffold stiffness and

cell morphology and to monitor cell behaviour by confocal microscopy. Now, they hope to find out how exactly mechanical stimuli influence the behaviour of normal bone cells and mesenchymal stem cells.

Towards clinical applications

What the surgeon needs is products that can be used off-the-shelf, preferably in a minimally invasive way, leading to the rapid recovery of the patient. This may sound like wishful thinking, but a lot of progress has already been made towards such products.

Growth factors like the various Bone Morphogenetic Proteins (BMPs) and their counterparts in cartilage are being tested in clinical trials. The first results suggest that they might be a valuable tool in the surgery of the spine. The development of smart scaffolds that can be injected or 'seeded' may yield even better and more cost-effective solutions. The search is on for scaffold materials with the optimal physical and chemical properties to attract repairing cells and circulating growth factors, eliminating the need to add cultured cells and synthetic growth factors.

Not all of this research is taking place within the DPTE programme, but thanks to the programme there is a great deal of synergy between fundamental and clinical researchers working to improve the mobility of our ageing population.

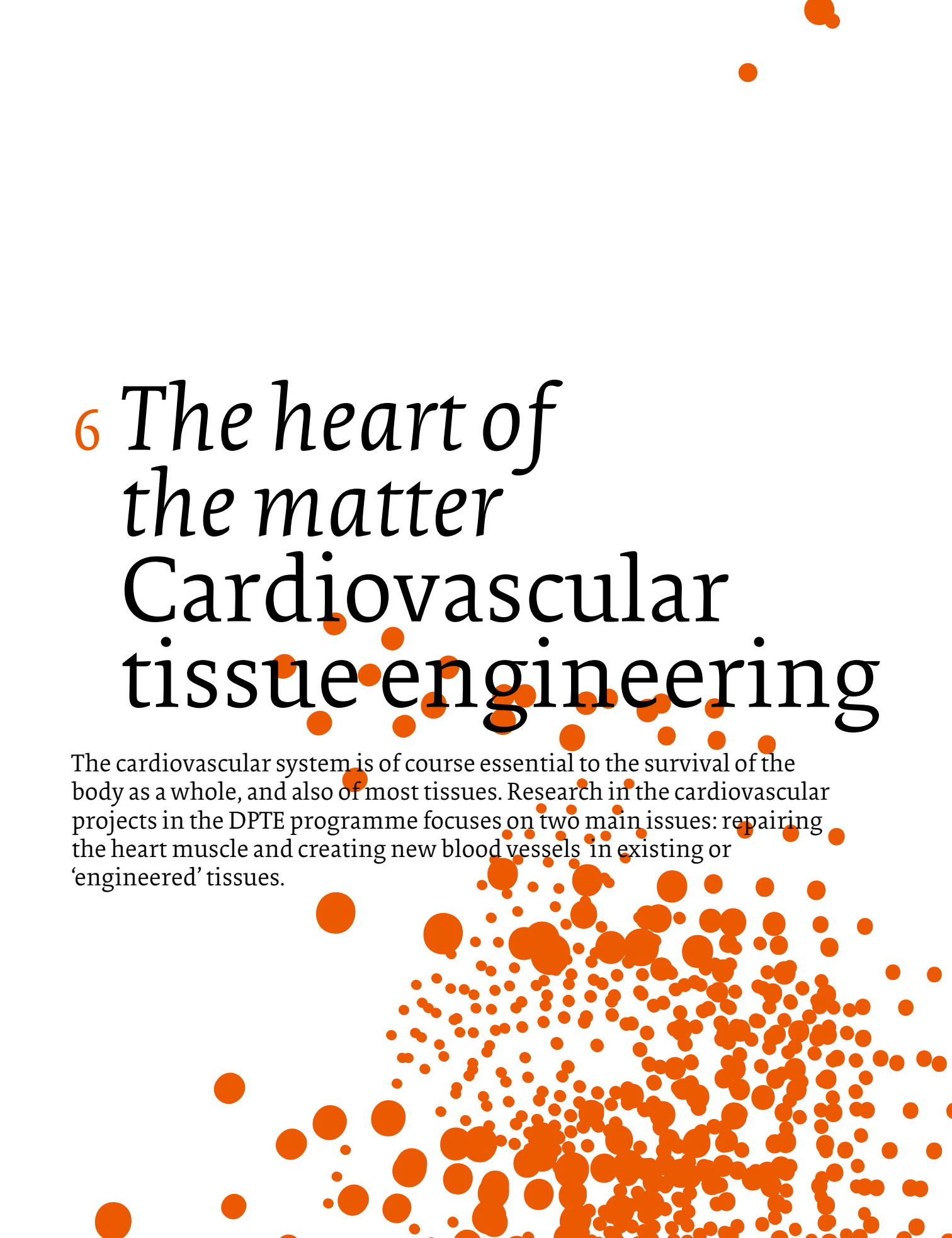
FULL-DEPTH LIVING SKIN

Researchers at the Vrije Universiteit Medical Centre (VUmc) in Amsterdam had just launched the company 'A-skin' when they joined the DPTE programme. The company produces and tests various products to cure skin defects. Their first product was Tiscover[®], a full-thickness skin substitute based on a culture of the patient's own skin cells on a cell-free donor dermis. It is used to treat chronic wounds. Clinical studies have so far yielded very promising results. It is thought that the amplification of skin cells in culture activates the cells, speeding up the formation of blood vessels (angiogenesis) and other processes essential to healing. Other products are being developed to accelerate the healing of surgical wounds and burns. Meanwhile, the fundamental research on skin tissue engineering continues. Much of this work is being done under the auspices of DPTE.

The skin substitute to cover burns is being developed in close collaboration with researchers at the Beverwijk Burns Centre. It differs from the usual skin grafts in that it is grown without animal serum and without so-called feeder cells from animal cell lines. Use of these animal products is now against European legislation. To optimise the 'take' of a skin graft in areas with poor circulation, it would be ideal if the lower layers of the graft (the dermis) were vascularised. Possibilities are being studied in collaboration with VUmc's Department

of Physiology (see also page 37). Another future development involves the use of synthesised scaffolds. For practical purposes, A-skin uses dermis from donor skin as the basis for the skin substitute Tiscover. Since the skin substitute is used only for relatively small patches, the donor skin shortage has not presented any practical problems as yet. Alternatives are being researched for the future, however. One involves the use of fibrin made in genetically modified cells (see page 37).





6 *The heart of the matter* Cardiovascular tissue engineering

The cardiovascular system is of course essential to the survival of the body as a whole, and also of most tissues. Research in the cardiovascular projects in the DPTE programme focuses on two main issues: repairing the heart muscle and creating new blood vessels in existing or 'engineered' tissues.

If the idea of engineering tissue grafts in the laboratory is to succeed, the grafts will have to be connected to the cardiovascular system of the host. Otherwise, the cells in the graft will not get the oxygen and nutrients they need. Providing adequate vascularisation is no easy task, however. It requires the growth of three-dimensional structures in a highly organised way. To accomplish this, biologists with their knowledge of the different cell types involved in growing a vessel will have to work together with tissue engineers who work on different types of scaffolds. Somehow, blood vessels will have to find their way within the three-dimensional structure of the tissue. To be able to guide them, one must know what signals are involved and under what conditions vascularisation is optimised.

Another approach focuses on cells of different origins. They, after all, have to do the job of building the new blood vessels. 'It's a fascinating world', says Prof. Victor van Hinsbergh (Institute for Cardiovascular Research, Vrije Universiteit medical centre, Amsterdam). 'Gradually we are learning how to make blood vessels grow exactly as we want them to grow.'

A low oxygen level stimulates the growth of blood vessels.

Other research within DPTE focuses on the repair of the 'engine' of the cardiovascular system: the heart itself. In this chapter, we look first at research on blood vessels and vascularisation. The final section discusses research on heart repair.

Building blood vessels

In the body, the growth of blood vessels is regulated by several factors. Research has shown that several growth factors are involved in vascularisation. They are produced by several types of cells, including the endothelial cells that form the lining of every blood

vessel, the platelets that are involved in blood clotting and several types of white blood cells. Another factor influencing the growth blood vessels is a low oxygen level (hypoxia).

Research on blood vessels within the DPTE programme has produced with several new insights into the interplay between different cell types and the influence of oxygen. Step by step, researchers are succeeding in building a toolbox for the engineering of vascularised tissues.

In the first half of the programme, a lot of energy has been invested in the development of a special infrastructure for research purposes. Now, researchers are able to study the interaction between different cell types in a realistic setting, complete with flowing blood. This setup also allows them to study the influence of several types of blood cells, including platelets.

Another interesting aspect that needs to be addressed is the effect of shear forces. As blood flows through a vessel, it exerts a force on the endothelium. This shear force appears to be important to the vitality and functioning of endothelial cells and may influence vascularisation.

FRAME 12

Conditions

To study the effects of the local environment on the formation of blood vessels, different approaches are being used. Evaluating the pattern of gene expression under different conditions, such as levels of oxygenation, reveals a lot of information about how specific intracellular processes are triggered. Together with chemical analysis of the tissues, this approach has afforded new insights into the effects of short-term and long-term lack of oxygen on vascularisation.

FRAME 13

Different cell types

On the cell biology front, DPTE has already yielded several exciting findings. A special subpopulation of mesenchymal stem cells has been developed that has several advantages over other stem cells. Cells of this special subpopulation are able to migrate through tissue – an essential property in the

‘We are learning how to grow blood vessels the way we want them to grow.’

formation of blood vessels. The researchers have found a way to culture these cells, keeping their migratory properties intact. They also have found that these special stem cells have the ability to stimulate the growth of human endothelial cells into capillary structures.

Sometimes, things turn out to be more difficult than previously thought. Some years ago, there was a lot of excitement at the discovery of endothelial progenitor cells (EPCs) in the bloodstream. The cells were thought to be able to differentiate into endothelium, helping the (re)vascularisation of tissues. However, closer study of these cells has shown that only a small number of them are actually able to differentiate into endothelium. The majority of the cells formerly labelled EPC have other origins, mostly different kinds of white blood cells (monocytes and lymphocytes). They probably play a supporting role in the generation and protection of newly-formed blood vessels. All the different cell populations are of course being extensively studied for their possible use in tissue engineering.

Scaffolds

Another highly productive approach to the formation of new blood vessels focuses on the scaffolds, the material that cells grow in. The knowledge gained in cellular biology can be used to provide the different cell types needed for vascularisation with the growth factors and other biochemicals that they thrive on. The physical properties of the matrix can also be taken into account. Some research focuses on the vascularisation of tissue grafts, by ‘seeding’ the matrix with endothelial cells, stimulating

Summary Many factors contribute to the development of new blood vessels in the body. Knowledge about the interaction between different cell types, growth factors and the matrix allows scientists to guide vessels through the tissue.

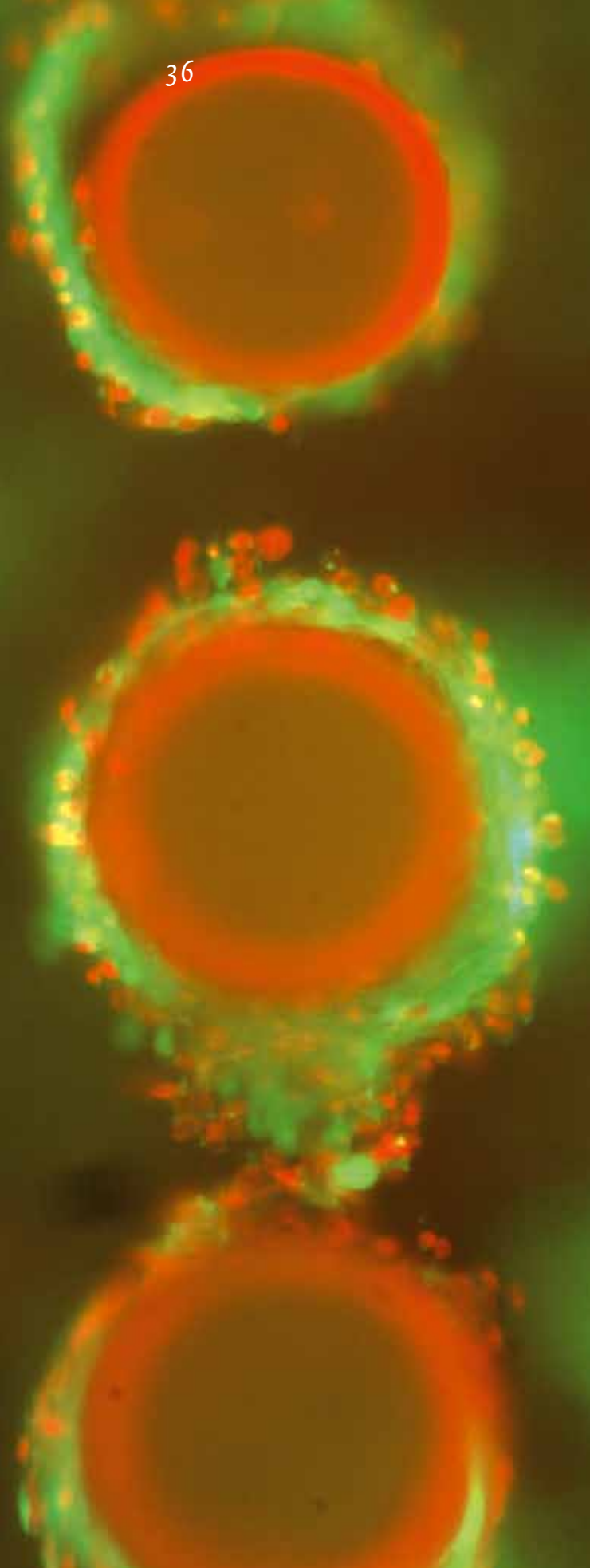
FRAME 12 PREPARING TISSUES TO HOLD THEIR BREATH

An infrastructure has been set up at the Laboratory for Physiology at the VU medical centre in Amsterdam to study the long-term culture of vascular cells and their progenitors under different oxygen levels. One of the advantages of this setup is that researchers can mimic the circumstances of early graft survival. After implantation into the body, the tissue graft will experience a temporary lack of oxygen caused by the absence of blood perfusion. With a little help from science, this may help to trigger the formation of new vascular structures. One of the hypotheses being tested is whether preconditioning of tissue grafts by lowering their exposure to oxygen can improve the survival and future perfusion of an engineered graft.

FRAME 13 HIGH CHOLESTEROL LEVELS INTERFERE WITH ‘BYPASS’ FORMATION

Oxygen levels are only one of the many variables that will influence the perfusion of graft tissues. Of particular interest are the levels of biochemical products that are likely to be elevated or lowered in patients receiving tissue grafts. That is why in vivo models are important, enabling scientists to study conditions like hyperglycaemia and hypercholesterolaemia.

Using these models, it has been found that hypercholesterolaemia, a high level of blood cholesterol, reduced the formation of collateral vessels. This has clinical implications both for tissue engineering and for the treatment of patients with cardiovascular diseases. Collateral formation (the spontaneous formation of a ‘bypass’) is important to preserve the blood flow if the main blood vessels are obstructed by atherosclerotic lesions. Reducing the cholesterol levels may contribute to collateral formation. In a tissue graft, vascularisation will be affected by high blood cholesterol levels, so measures must be taken to lower them.



them to grow into tubular structures. A slight modification of the building blocks of the matrix has been shown to influence vessel growth.

FRAME 14

Blood vessels themselves can also be grown and implanted, to bypass obstructions in vital arteries. To accomplish this feat, DPTE researchers have created tubular scaffolds based on two proteins that are prominent in connective tissue: collagen and elastin. These tubes are then enriched with molecules that enhance the binding and development of cells. The scaffolds act as anchors for endothelial and smooth muscle cells, the major cell types needed to grow a complete blood vessel.

Regeneration of heart muscle is a major challenge.

Repairing the heart

Thanks to advances in cardiology, an increasing number of people are surviving the consequences of a myocardial infarction. But if part of the heart muscle has been damaged, it is gone forever. Scar tissue comes in its place, keeping the heart intact, but the damaged part will never again contribute to the circulation of blood through the body. As the damaged heart ages, it progressively loses the capacity to meet the demands of the body, resulting in so-called heart failure. With proper medication, much can be done to improve the quality of life of these patients, but until now it has been impossible to regenerate lost heart muscle.

This is the challenge for scientists working on heart muscle cells (cardiomyocytes) and their progenitors and stem cells. Using grants from both DPTE and the ICIN (Interuniversitair Cardiologie Instituut Nederland) they are aiming to develop functional heart muscle tissue. This means that the cardiomyocytes not only have to live and contract, but that they also have to contract in a coordinated

way, obeying the electrical signals that regulate the beating of our hearts. Several important steps have already been taken on this road. Human cardiomyocyte progenitor cells have been isolated from heart muscle tissue and pericardium. Scientists were able to culture them and make them differentiate into cardiomyocytes. The cells were further characterized and their contractile and electrophysiological properties investigated. The cultured cells displayed spontaneous and rhythmic contractions, showing that they have the potential to become a functional part of the heart.

Using embryonic stem cells, researchers were able to construct rings of cells with cardiomyocyte properties. These rings were able to contract in a rhythmic and coordinated way. Electrical coupling, which makes our heart muscle contract in the right rhythm, seemed to be present. In the future, it is conceivable that rings of artificially grown heart muscle could be applied as a functional band aid on a patient's heart, supporting the contraction of the heart and thereby repairing the loss of function.

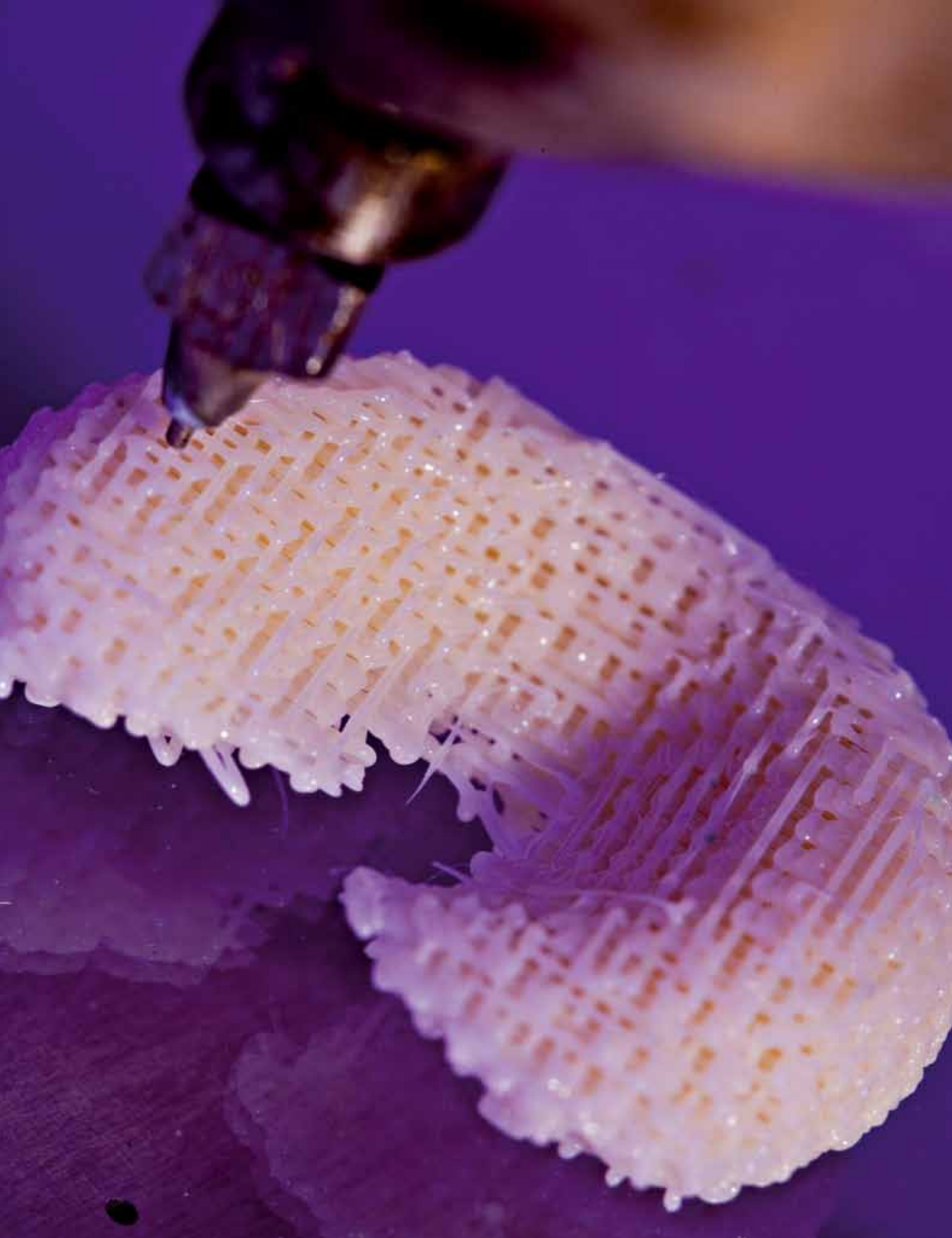
Animal studies have shown that it is possible to restore cardiac function using such an approach. In mice, an artificial myocardial infarction was induced. Then, human foetal cardiomyocyte progenitor cells were transplanted into the animals. Two weeks later, their cardiac output had significantly improved. Although it may be a long way from mice to men, these results are an encouraging step towards clinical applications of this technology.

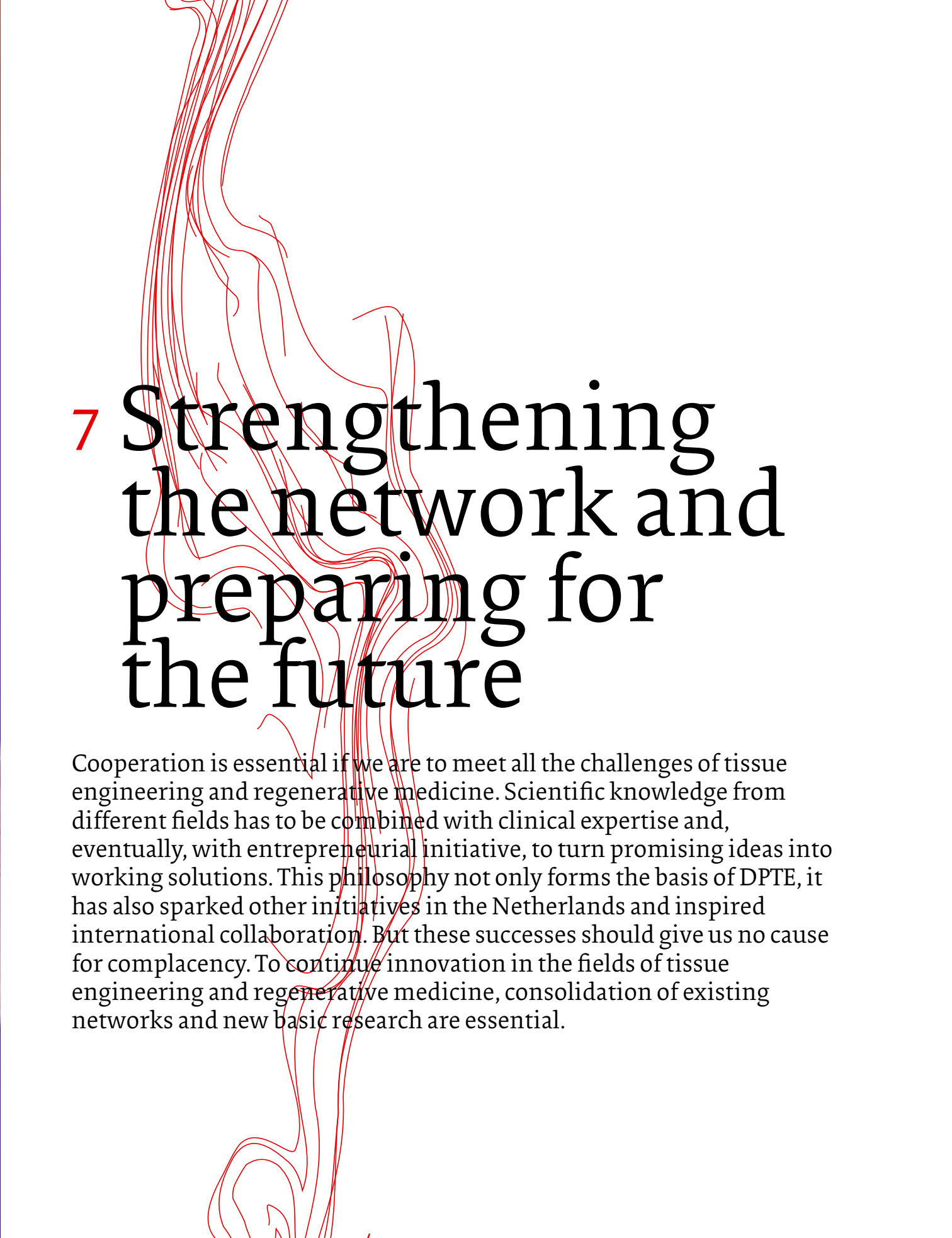
Summary Heart muscle tissue does not have the ability to repair itself. To heal the growing number of patients suffering from heart failure, new ways are being investigated to stimulate the growth of functional heart muscle cells. They will have to contract in accordance with the electrical signals in the heart. Basic research offers promising clinical perspectives.

FRAME 14 GUIDING BLOOD VESSELS THROUGH THE TISSUE

One of the materials used as a scaffold for different types of tissue is the blood clotting protein fibrin. It exists in two forms, a long molecule and a slighter shorter version. The long molecule has been found to facilitate vascularisation, while the shorter one slows down the growth of blood vessels. This knowledge, which can be used to guide vessels into the desired direction, has led to a patent application.

A lot of progress has been made in the field of skin transplants. Using fibrin as a matrix for vessel ingrowth, improvements have been made in the vascularisation of complex skin transplants. Now, research focuses on optimising the depth of blood vessel penetration.





7 Strengthening the network and preparing for the future

Cooperation is essential if we are to meet all the challenges of tissue engineering and regenerative medicine. Scientific knowledge from different fields has to be combined with clinical expertise and, eventually, with entrepreneurial initiative, to turn promising ideas into working solutions. This philosophy not only forms the basis of DPTE, it has also sparked other initiatives in the Netherlands and inspired international collaboration. But these successes should give us no cause for complacency. To continue innovation in the fields of tissue engineering and regenerative medicine, consolidation of existing networks and new basic research are essential.

DPTE has been successful in terms of scientific progress, as the preceding pages have shown. Perhaps even more important has been its success in terms of cooperation between different scientific institutions and others, especially smaller and larger companies. This network of enthusiastic collaborating groups will no doubt produce even more tangible results in the remaining three years of the DPTE programme. It has already generated new initiatives, attracting the necessary funding for scientific and commercial activities. One of the most important outcomes of DPTE has been the formation of networks between scientists in different fields. People have come to know each other well, as a direct result of our activities. Also, the basic scientific knowledge about stem cells, scaffolds and bioreactors generated within DPTE has been a driving force towards other, more application-oriented initiatives', says Prof. Jan Feijen, Professor in Biomaterials and Polymer Chemistry at Twente University (Enschede) and chairman of the DPTE.

The cooperation within DPTE has sparked other initiatives.

In this final chapter, we briefly discuss these developments and their impact on the field of tissue engineering and regenerative medicine. And, of course, we take a look at future developments.

DutchFoRM

One initiative that can be said to be a direct result of network building within DPTE is the Dutch Forum for Regenerative Medicine (DutchFoRM). It was initiated by ZonMw, STW and a group of enthusiastic scientists as a platform for research, development and education, and as a way of raising public awareness of regenerative medicine. It

involves several Dutch companies, universities, university medical centres and patients' organisations. All participants have committed to working on several issues associated with regenerative medicine over the coming years. Regenerative medicine includes tissue engineering, but the term is also used to cover interventions that aim to improve the body's own repair mechanisms. This approach may eventually reduce health care costs, eliminating the need for expensive replacement surgery. It may also help reduce the secondary costs of disease and disability. Another major objective of DutchFoRM is to capitalise on the know-how in the Netherlands and generate economic activities connected to scientific and clinical results.

DutchFoRM itself has no budget to finance scientific or other activities, but it supports participants in their quest for adequate funding. One major success has been the recent SmartMix application (see below).

SmartMix TeRM programme

A consortium of companies and scientific groups, most of which are also involved in the DPTE programme and DutchFoRM, has successfully applied for a SmartMix grant. The relatively large grants available under this scheme – an initiative of the Dutch Ministry of Economic Affairs and Ministry of Education, Culture and Science – aim to create enough focus and mass to really make a difference in terms of economic activities and addressing the needs of the public. It covers many areas, including health care.

The programme on regenerative medicine, known as TeRM (Translational excellence in Regenerative Medicine) promises to 'translate technology into clinical practice'. It focuses on tissues that are also the subject of research under the DPTE programme: bone, cartilage, heart and blood vessels. The aim is to perform the necessary translational research to make the results of laboratory research ready for clinical practice and to conduct clinical trials to demonstrate the effectiveness of new technologies. Working with several companies, some of them

spin-offs of university research groups, scientists hope to create products that will improve the health of people all over the world, and also boost the Netherlands' 'knowledge-based economy'.

BMM

The BioMedical Materials programme (BMM) is a 'top technological institute' (TTI), where leading scientists and major industrial players cooperate, together with other partners (small and medium-sized enterprises (SMEs) and non-profit organisations), working on innovations in a promising field. Other TTIs in the Netherlands are, for instance, working on polymer technology, pharmaceutical innovations, translational molecular medicine, metals, water and telematics. Some of these TTIs, particularly the Pharma Top Institute (TIP) and the Centre for Translational Molecular Medicine (CTMM), may produce results that will also contribute to the success of tissue engineering and regenerative medicine. TIP may contribute to the development of drugs that help to regenerate tissues and protect implants. Technologies developed within CTMM could make several contributions to the field of tissue engineering, for instance in the monitoring of tissues growing in bioreactors.

Biomedical materials play an important role in tissue engineering, with a particular focus on 'scaffolds' (see Chapter 3). BMM, which will soon receive funding from the Dutch government, promises to establish 'breakthrough innovations' and will translate them into successful medical applications, intellectual property rights, and scientific publications. It will work on the same subjects as DPTE as well as on other new developments in tissue engineering. With this focus on practical applications, the BMM programme has the following goals:

- to make therapies less invasive and painful
- to reduce the need for revision surgery
- to reduce the side effects of medical treatment and
- to shorten recovery times.

Summary The DPTE network has been the cradle of several other initiatives, such as the Dutch Forum for Regenerative Medicine (DutchFoRM). A large grant ('Smart Mix') is used to develop promising scientific results into healthcare products. The BioMedical Materials programme (BMM) also unites scientists and companies working towards practical applications.

MORE INFORMATION

More information on the various initiatives mentioned in this chapter can be found online at:

ZonMw: www.zonmw.nl

STW: www.stw.nl

DutchFoRM: www.dutchform.org (in Dutch)

BMM: <http://www.biomedicalmaterialsprogram.nl>

SmartMix: www.smartmix.nl

SmartMix TeRM: http://www.bmti.utwente.nl/Nieuws/term_4o.doc/



International cooperation

Modern science is driven by international competition and cooperation. The high level of cooperation and organisation within the Dutch field of tissue engineering and regenerative medicine gives the various scientific groups in the Netherlands an edge over the international competition and makes them attractive partners for successful groups in other countries. Many groups working in the DPTE programme and the other initiatives mentioned here also have projects under one or more European Union Framework Programmes. Exchange of scientists, knowledge, procedures and materials occurs with scientific institutions all over the world. There is an interesting paradox here: strengthening the infrastructure and cooperation on a national level has proved to be an effective way of stimulating international interaction, yielding more scientific and clinical results. In the end, it will be patients who benefit.



DPTE THE YEARS AHEAD

DPTE was originally intended to end in 2009. However, the Dutch government recently agreed to extend the programme and the necessary funding to the end of 2010. DPTE chairman Prof. Jan Feijen: 'In the beginning we lost almost a year in our original planning, due to factors beyond our control. Now we have enough time to complete our programme and prepare for the future.'

So what about the future? First of all, the work described in the previous chapters will continue. This will yield many scientific and practical results. According to Feijen, one special focus will be the generation of patents. 'Though this is not, of course, a goal in itself, it is a necessary first step towards practical applications.'

Meanwhile, Feijen and other Board members are already thinking about a second DPTE-like programme. There is a broad consensus within the field that such a second programme will be essential to maintain the momentum that has been created. This was also the opinion of an international evaluation committee, reporting on the first half of DPTE. They wrote: 'Research groups from all over the country interact in a joint interdisciplinary programme. Such a programme is quite unique in Europe. DPTE is clearly on its way to position The Netherlands at the frontier of tissue engineering. The Evaluation Committee thus unanimously gives a positive evaluation which justifies a continuation of the DPTE programme'. Feijen adds: 'Other initiatives, like Smart Mix TeRM and BMM, are now building on the scientific results of our current programme. They will help to translate the science into practical applications. We now have to

prepare for the future. Further basic research is needed to generate the next wave of applicable outcomes. Of course, when we prepare for a second programme, we will take all these other activities into consideration. We have to find a good balance between basic research and clinical breakthroughs. In my opinion, we will need to invest more in basic research, so we can lay the foundations for future innovations.' The discussions are at a very early stage, so nothing conclusive can be said about the contents of any follow-up programme. Feijen sums up some new themes that might be included, however. 'When we prepared the current DPTE programme, we very much regretted that there was no room for several promising research lines. Research on the regeneration of nerve tissue is, for instance, in my opinion a very interesting subject. It holds the promise of functional recovery for patients with paraplegia due to spinal injuries and patients with peripheral nerve damage. Another area that might be included in a future programme is research on the generation of functional organs like the liver or kidneys. There is a growing shortage of donor organs for transplantation; artificial living organs could be a solution. But a lot of basic questions have to be answered before

we can even begin to grow these organs in the laboratory.'

Whatever its exact contents, a second programme will certainly build on the achievements of the current DPTE programme. The networks that have been established between scientists working on stem cells and their colleagues studying scaffolds and bioreactors will continue and perhaps even be strengthened. Feijen mentions a promising new scientific discovery concerning the 'reprogramming' of common body cells (fibroblasts) to form stem cells. This would open up new possibilities, for it would provide scientists with an almost infinite source of stem cells for practical applications. Together with 'smart scaffolds', our growing knowledge of how to monitor tissues in a bioreactor will open up a whole new range of tissue engineering possibilities in the near future. Dutch scientists, cooperating with each other and with partners in industry and other organisations, are ready to make major contributions to this exciting field.

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