The book was published to coincide with the 20th anniversary of the Zbigniew Religa Foundation of Cardiac Surgery Development.

IMPLANTEXPERT edited by Zbigniew Nawrat

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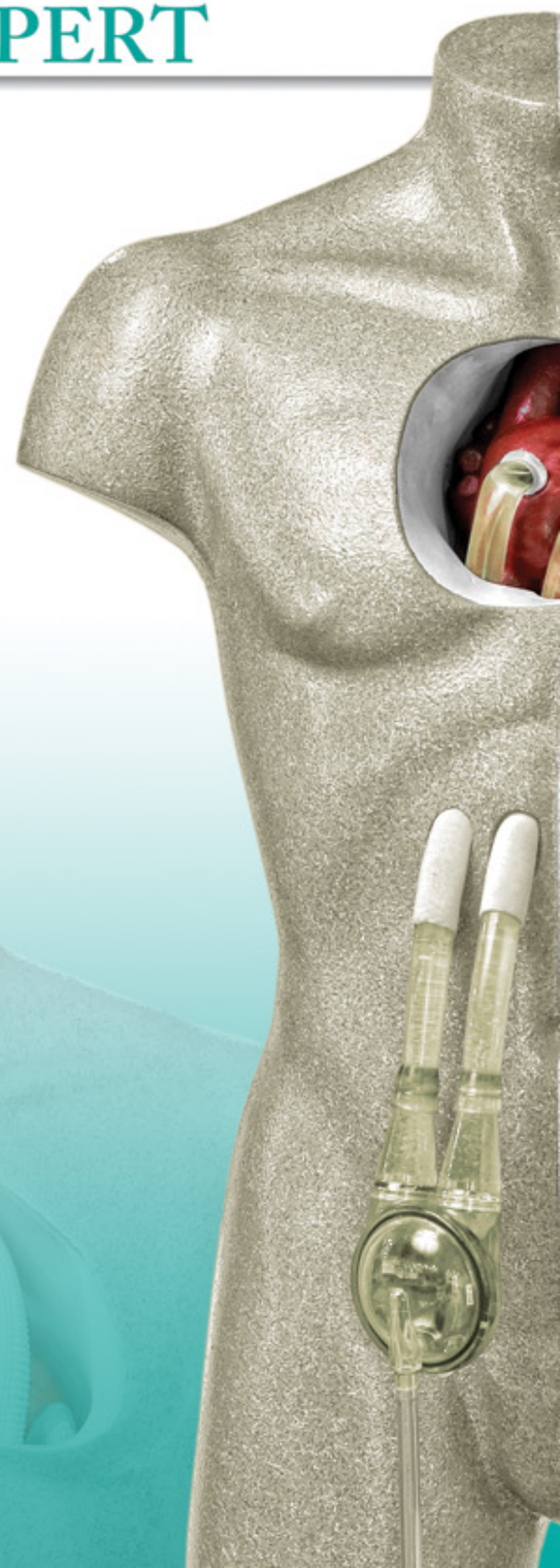

ImplantExpert

edited by Zbigniew Nawrat

This book is dedicated to the memory of Professor Zbigniew Religa - a great doctor, visionary and teacher.

Zabrze 2011

ImplantExpert
edited by Zbigniew Nawrat

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Dear Reader,

This book is devoted to Polish achievements in the field of research and development in the bioengineering of artificial organs of the circulatory system. It is a very important book for me, my colleagues and friends of the Foundation of Cardiac Surgery Development, which this year celebrates its 20th anniversary.

The creation of this book was triggered by the need to sum up two research projects involving explanted biomaterials and artificial organs. Thanks to our participation in the European COST 537 project, and then the activation of a Polish research project on explanted circulatory system materials and the organisation of a network of laboratories, today, we are leaders and promoters in this field. I hope that this monograph will serve you not only as a source of expert knowledge on artificial organs and biomaterials, but also as an inspiration towards your own research and development.

It is a collective work showing a spectrum of Polish activities involving artificial organs, especially the heart and the circulatory system. You will also find historical references – attempts to place events on a timeline of Polish advances in this field. The book concludes with a reflective chapter on the ethics of artificial organs and modern medicine.

I dedicate this book to Professor Religa, the Master of the entire New School of Cardiac Surgery in Poland, but also Professor Religa the Man, Doctor and Boss, who paved the way for the co-operation between specialists of the once so distant fields of technology and medicine. The interdisciplinary team created and led by him, continues to introduce new devices into clinical practice, shoulder to shoulder with doctors, and by the patient's side.

The artificial heart, prosthetic heart valves, biomaterials, modern advisory systems and now also robots and other minimally invasive surgeon's tools are the result of works carried out by the Foundation of Cardiac Surgery Development created by Professor Religa in Zabrze - a city renowned for a major breakthrough in Polish medicine: the first successful heart transplant. Professor Religa did the impossible to create the Polish circulatory system prostheses. Right until the end, it was his dream, passion and obsession.

The authority of the Professor and his team, the open promotion campaign and the introduction of growing numbers of young scientists into the works on the artificial heart, heart valves and the Robin Heart robot, gave the spark

that ignited interest in bioengineering in Poland, restoring belief in the proverbial “a Pole can do it”.

Although he is no longer with us, we still follow the path he set out. The irreplaceable Professor changed us all for the better, giving us faith that together we will be able to fulfil His Great Plan.

I trust that you also, dear Reader, will find hope and inspiration in this book.

Zbigniew Nawrat
Zabrze, August 2011

Acknowledgements

I would like to thank Joanna Mikusz for her never-fading patience and professionalism in providing top quality DTP services. I also wish to thank Sandra Lindon for the translation of selected chapters and painstaking proofreading of extensive parts of this is book. Great thanks to Mariusz Jakubowski for the beautiful photos and book cover design.

Zbigniew Religa - an unfinished story

Zbigniew Nawrat
Medical University of Silesia
Foundation of Cardiac Surgery Development

It is with great sadness that we received the news first of Michael de Bakey’s, Adrian Kantrowitz’s, Peer Portner’s, Willem Kolff’s and now Zbigniew Religa’s death. The picture of the passing generation of pioneers is complete. But our Masters will always be with us, and their places at the forefront shall remain empty. Their absence has irrevocably changed our world. At the same time, it has imposed on us a new obligation – to ensure that their work will not go to waste.

Great teachers

The great teachers knew and respected one another. They cooperated, shared their knowledge and experience. Portner and Kantrowitz visited our Foundation of Cardiac Surgery Development, where they became acquainted with the Polish artificial heart. We could always count on their kind advice and experience. We met de Bakey and Kolff at numerous conferences. I will always remember Kolff’s presentation at the XXVIII Congress of the European Society of Artificial Organs in Gandava. He mesmerised us with his intrepidity and unconventionality taking out of his briefcase two kettles and an apple as his scientific aids. At the turn of the century, the century of silicon, genes, tremendous computational powers and computers, with a playful glimmer in his eye, he would explain to the audience the workings of his prototype artificial kidney or how to construct an artificial heart using half an apple. I video recorded his lecture, and later sent it to him and some friends. To this day, I show parts of the recording to my students of the only medical course in artificial organs in Poland, as I want them to see one of the heroes who had changed the world and medicine. The act of creation is startling as it combines intuition, knowledge and the ability to learn from mistakes. Kolff used to repeat that the values we should strive for are the patients’ joy of life, and their return home. Kantrowitz, the founder of the American School of Transplantology, was the teacher of Christian Bernard, who eventually excelled his master by performing the world’s first human heart transplant operation, and of Zbigniew Religa, the father of Polish heart transplantology. In the 1970s, Zbigniew Religa was a chief resident taking care of patients with mechanical heart support (a dynamic aortic patch working as a permanent mechanical auxiliary

ventricle, so-called “Kantrowitz patch”). The Kantrowitz family were charmed with this bright and handsome Pole who worked with complete devotion and knew to treat women with Slavic gallantry. Religa had become a paragon that many young doctors, successively arriving in the USA to study and practice, found hard to match. Despite tempting job offers, Zbigniew Religa came back to Poland to lay the foundations of modern cardiology, from transplantology to the artificial heart. He came back to show that a forward-looking physician, working arm in arm with engineers and biologists, may save the lives of even the most difficult of patients. He will be remembered as the founder of Polish transplantology and the Polish human heart. He was a great innovator- the first Polish doctor who was brave enough to transplant a pig heart to a dying patient- and a promoter of Polish surgical robotics.

Adrian Kantrowitz

Born in New York, Kantrowitz performed the world’s first paediatric heart transplant, invented and implemented the intra-aortic balloon pump and developed his own, original heart assist device (Kantrowitz patch).
In the course of his long career, he invented over 20 electronic and medical devices for patients with heart diseases and paraplegia. In the 1950s, he cooperated with his brother Arthur, a physicist and founder of the Avco-Everett Research Laboratories. Finally, in 1966, Kantrowitz was ready to perform a heart transplant operation. The approval was obtained from the Ethics Committee and the infant donor’s parents; unfortunately, the delay in harvesting the donor’s heart, resulted in its damage. This true pioneer performed over 400 heart transplants in small animals. And yet, it was his disciple, Christiaan Barnard from South Africa, who performed the world’s first successful human heart transplant operation.

In 1972, the first patient returned home after hospital treatment involving a heart-assisting pump; unfortunately, he died 3 months later. Nevertheless, by propagating heart-assisting devices, Kantrowitz was far ahead of his time.

Until his last days, Kantrowitz worked on improving his system. When in November 2008, at the age of 95, he was on his deathbed, the FDA commission was considering another of his heart-assisting systems – a new model of a heart pump, manufactured by LVAD Technology Inc. of Detroit (a company which he had founded and run together with his wife). The system is currently undergoing clinical tests.

Jean Rosensaft, Kantrowitz’s wife, has Polish roots and, following our arrangements, visited the place of her family’s origin. They have three children, all of whom are doctors. Kantrowitz was a keen motorbike rider, and he also piloted his own plane.

Zbigniew Religa

Professor Religa was my boss for over 20 years. Not being a doctor but a physicist, I am proud that such a renowned surgeon always treated me as a partner.
He was an unusual person: a father figure for many doctors fascinated by modern surgery, a physician and creator of medical innovations, always open to interdisciplinary collaboration and, last but not least, a great organizer of ventures that have become crucial to the advancement of medicine. He turned down a lucrative job offer in the USA to create the foundations of modern Polish cardiac surgery. Chairing one of the Departments of the Silesian Medical Academy, he founded the Artificial Heart Laboratory. When this proved insufficient, he established the Foundation of Cardiac Surgery Development, where he created the only Polish non-commercial and non-governmental research institute working on materials and devices, such as heart prostheses, heart valves, surgical robots and bio-technology.

With his unstoppable care for the difficult patient, he won the hearts and loyalty of his co-workers and earned his excellent reputation. He initiated heart transplants in Poland and implemented an original Polish system of mechanical assistance of the human heart (POLCAS). He did not hesitate to implant a pig’s heart to a hopeless patient. He also used thymus-derived preparations as an original method of defence against the effects of fast reject reaction. He developed several prototypes of heart valve prostheses and implemented numerous surgical procedures that were revolutionary for the Polish conditions of that time. In his pursuit to modernise the healthcare system, making it efficient and available to all, he became a Member of Parliament, a Senator, and finally Health Minister.

With his great knowledge and experience, combined with extraordinary courage and devotion, he won many faithful hearts of his colleagues and friends. He changed me, changed us all, for the better. His way of building a team was based on respect for individuality. The rule was: as much independence as responsibility. This was a “deep water test”.

We always had the impression that everything depended on us. Accordingly, we worked to gain the skills and expertise that he required from us, sometimes unique in Poland, as we knew that the future of our patients, the future of the

Cause, was in our hands. He was always there, ready to help, offer advice, assume responsibility; but to us, it just did not seem right to ask. This way we matured and grew to believe in ourselves; his objectives became ours.

His **Patient-based** Code shall always remain in our memory. He would travel hundreds of kilometres, work days and nights, if there was as much as a glimmer of a chance to save a single human life.

He created the **System** to save patients. Zbigniew Religa not only overcame the barriers of modern medicine, but also created the organizational frameworks for many successive projects, guaranteeing their continuation, durability and further development. He knew very well that although the first step is important, continuous improvement is equally relevant, especially if accompanied by the advancement of technology. Because it is possible to change the world and because our patients deserve the best they can get. These goals should be the objective of civilizational progress. Not only did he perform the first human heart transplant, but also ensured the creation of a Polish transplantology programme. Until his last days, he supported the National Artificial Heart Programme, which will enable the development of a modern, comprehensive heart assisting system saving the lives of patients irrespective of their age and health condition.

The **Team** played an important role in Religa's Code. Our success was his success. And his success was ours. He always emphasised our participation in His ventures. He knew better than anyone else that science, medicine, is all about team-work. And, as in football, his favourite sport, the final result depends on the participation of every team member, and is never certain. Failure is just a motivator to undertake a more effective fight next time.

Religa's Code includes **Poland**, duty and organic work, with pride, dignity and devotion.
When a hero passes away, everything comes to a momentary halt but then goes on, as if nothing had happened. And we are just left bewildered by the number of empty places.

We will always remember Professor Religa as an amazing and extraordinary personality, offering bunches of flowers to his beloved wife, walking his dog Bamba along the Vistula River bank, rod fishing, supporting the football "Górnik-Zabrze Team", taking his grandchild to football matches, teaching his own children integrity, running for Presidency. He was John Wayne of Polish

medicine and politics. A man of flesh and blood. He trained boxing, although he had wanted to be a philosopher. He thrived on challenges, and was always ready to face problems with courage. Some of his opponents were apprehensive of his granite-like personality. He was loyal to his friends. Towards women, he was always a perfect gentleman. He knew how to talk to people, understood human problems and joys. He was adored by his patients. He was impeccable in handling the media. In just few seconds he was able to sum up any message, be it a patient case study or an act of law debated in Parliament.

I talked to him in January 2009 during experiments on animals with the Robin Heart Robot. While we were operating on the heart, I was holding the endoscope in one hand and the telephone in the other, relating to the Professor all the details of the operation. **Until his last days he was deeply involved in our work, and a lot of his advice we have yet to follow. The challenges he set forth are still valid.**

The last time I saw Professor Religa was in the Presidential Palace where he was awarded with Poland's highest state distinction - the Order of the White Eagle. He did not want any media coverage of the event, as if sensing that it was going to be a farewell to his friends and colleagues. He held himself straight and proud but no one knows just how much effort it cost him. Probably, he would have been swept away by his angels there and then, if it had not been for his incessant curiosity of this world.

An unfinished story

Our Masters have taught us that the story never ends. Let us not fail them!

Zbigniew Nawrat, D.Sc. in Medical Sciences, M.Sc. in Physics, is Professor Religa's successor at the post of Director of the Institute of Heart Prostheses, Foundation Cardiac Surgery Development in Zabrze. He is Assistant Professor at the Faculty of Cardiac Surgery and Transplantology, Medical University of Silesia, where he runs the only in Poland course in artificial human organs. He is the father of the Polish Robin Heart Robot.

But, none of this would ever have happened if it had not been for Professor Zbigniew Religa

Extensive excerpts of this chapter have been published in Artificial Organs: Z. Nawrat: ***"Zbigniew Religa: An unfinished story".*** The International Journal of Artificial Organs. Vol. 32/ No.6, 2009 June, pp. 315-317
REPRINTED FROM INT J ARTIF ORGANS 2009; 32(6); 315-317, WITH PERMISSION OF WICHTIG EDITORE

TABLE I - ZBIGNIEW RELIGA' S ACHIEVEMENTS

As shown below, Prof. Religa and his team (Figs. 2 and 3) achieved milestones in Polish cardiac surgery and heart transplant.

- **1985** - the beginning of the heart transplant program, leading to the first successful heart transplant in Poland,
- **1986** - the beginning of surgical treatment of early heart coronary condition,
- **1986** - the first heart and lung transplant in Poland,
- **1986** - the first use of heart-assistance pumps and the artificial heart (in cooperation with Vascu and Shumacov) in Poland,
- **1988** - the establishment of the first Polish Heart Valve Bank (deep freezing),
- **1989** - the first xenotransplantation (pig heart),
- **1990** - the first operation on chronic pulmonary embolism,
- **Since 1991** - founder and member of the Polish Transplantation Society.
- **Since 1992** - member of the Scientific Board of the Polish Ministry of Health,
- **1993** - founder of the Foundation of Cardiac Surgery Development,
- **1993** – the first implantation of the Polish Ventricular Assist Device (POLVAD),
- **1993** - founder of the Institute of Heart Prosthesis at the Foundation of Cardiac Surgery Development,
- **1993-1997 and 2001-2005** - Senator of the 3rd and 5th term in the Senate of the Republic of Poland,
- **2005** - candidate for President of the Republic of Poland,
- **2005-2007**- Minister of Health,
- **2007-2009** - Deputy of the 6th term of the House of Representatives of the Republic of Poland.

Pofessor Religa – the great surgeon: on the left –the first heart tranplant performed in Zabrze on 5.11.1985 by Z. Religa. At the operating table of the recipient, a 62-year old patient from Krzepice, on the left – A. Bochenek, on the right - J. Wołczyk; in the foreground: E. Kucewicz. By the donor's side: M. Zembala and B. Ryfiński (photo by S. Jakubowski).
Bottom: The Clinical Team after the first successful heart transplant (1985). From the left: Z. Religa, B. Ryfiński, B. Kominek, P. Czerwińska-Dzieman (nurse), A. Bochenek, J. Wołczyk, E. Łubek-Wilczewska (nurse.) and R. Cichoń (photo by S. Jakubowski).
In the top centre: After the first mitral valve disease surgery performed on 15.08.1985; leaving the operating theatre , from the left: M. Zembala, Z. Religa and W. Sitkowski;
The doctors and nurses of the Cardiac Surgery Clinic in Zabrze after the first mitral valve disease performed the same day. In the centre: Z. Religa, to his right: A. Bochenek and B. Czech, to his left: K. Suwalski and M. Zembala, lying: W. Sitkowski.











Professor Religa's Guests – from top left: Michele Legrand with sister and Grażyna Torbicka, hostess of most Heart for Heart concerts, Professor Religa and politicians: Jacek Kuroń and Lech Wałęsa (in the centre). Below, great scientists: Donald Ross, Andrzej Bochenek, Michał Wojtalik, Yukihiko Nose and Kou Imachi.

Professor Religa at the Foundation – the founder and chairman of the Foundation of Cardiac Surgery Development, surrounded by his colleagues; and at the Heart for Heart concerts.

Professor Religa at galas: receiving the Order of the White Eagle in the Presidential Palace and at the Heart for Heart concerts.

20 years Foundation of the Cardiac Surgery Development

by Jan Sarna

In 1991, Zbigniew Religa, then a university Reader, one of the most renowned Polish surgeons and a heart transplantation pioneer, was invited to Żywiec by a group of local doctors. In conversation, he mentioned his dream of creating a biological heart valve. However, in the face of the meagre economic situation of the national healthcare system, the obtaining of financial support to conduct the necessary research was science – fiction. By chance, this was overheard by the chairman of the Żywiec Brewery who immediately offered his help. But then a question arose: what account should the money be sent to? The hospital's, the clinic's, the medical academy's or to a private account? The creation of a foundation seemed to be the best solution. Professor Religa, who thrived on challenges, embraced the idea with fervour. In the autumn, he invited me to his flat in Reymont Street in Zabrze. I did not know what he wished to discuss but in my wildest dreams I could not have foreseen how that conversation would have influenced my professional career. At first, I thought we were going to talk about support for the clinic, and specifically about obtaining new equipment or medication for the patients. But as Professor Religa ardently kept describing his ideas for innovative scientific projects, such as the human living heart valve and artificial heart, it began to dawn on me that what he meant was an absolutely exceptional venture. I also began to see that he was much more than just a distinguished surgeon - the operating theatre was not enough for him and his passion led him to set out his own innovative visions and ambitious goals. I realised, that he was a pioneer in need of somebody who would implement those visions into practice. Then, he suggested the creation of a foundation that would put such audacious scientific projects into practice. He listed the aims and tasks that the foundation should fulfil, and our conversation continued until the small hours of the next morning. So efficiently did he design the foundation in that one night, that it is still successfully functioning today.

And so the Foundation of the Cardiac Surgery Development was created as a result of Professor Religa's visionary inspiration, under his supervision and with the good will of people who believed in the mission of introducing the latest life-saving methods into clinical practice. Officially, the Foundation was created by 17 people – doctors, managers and businessmen. I was responsible

for its organisational side, with its legal, financial and personnel aspects. Our beginnings resembled a true levy in mass: we began with the very modest means of 4.200 zł collected among the founders, one small room and basic office equipment brought from home. But from the very beginning we boasted the great capital of the “Zbigniew Religa trademark”, the scientific expertise in heart transplantation of the Cardiac Surgery Clinic in Zabrze and the painstakingly designed research programmes involving a new type of biological heart valve with an artificial heart. We also had high qualifications, great enthusiasm for work and a common belief in success.

Never at that time did I expect the Foundation to become what it is today. I did not expect that it would employ approximately 80 people and that scientific research would be conducted in our own, unique on a world scale, Institute of Heart Prostheses created in 1993 with over 2 million USD. I also did not think that both the Institute and the Foundation's headquarters, with a modern symposium hall, would be housed in a building that, since 1997, has been our property.

Today, after 20 years of activity, the Foundation for the Development of Cardiac Surgery is an innovative hi-tech institution functioning in compliance with the Quality Management System ISO norm 9001:2008. It is a public benefit organisation, a participant of numerous technological platforms, the owner of countless patents and the winner of a number of national and international prizes. The Foundation co-operates with international centres and participates in European projects. It organises its own scientific workshops, conferences and symposia. It also offers a cardiac surgery scholarship system for candidates from Poland and abroad. Over 200 national and international specialists enhanced their professional qualifications within the programme: groups of doctors form Georgia, Belarus and the Ukraine as well as scholarship holders from the Czech Republic, Holland, France, Germany, Argentina and the United States.

Without a doubt, the Foundation's greatest strength and achievement has been its scientific research, accompanied by the introduction of new ventures. The Foundation's main interests encompass the Polish artificial heart project, the cardiac surgery robot with new-generation robotised surgical tools, therapeutic tissue engineering and biological heart valve prostheses for the repair of congenital and acquired heart defects in children and adults.

One of the main research areas is the creation of a biological heart valve model with the recipient's own cells and based on the solutions offered by medical

biotechnology and tissue engineering. Placing great hopes in bioengineering and biotechnology, the Foundation engages in works with the use of stem cells for myocardial regeneration as well as the creation of nanostructural biosynthetic materials for medical purposes.

Since 2000, the Foundation has been conducting a unique project, aiming at the creation of a cardiac surgery robot family - the Robin Heart. Within this project, three different robot models have been created, as well as an ergonomic control console model and an independent robot controlling the position of the endovisual path, with a wide field of application in the majority of minimally invasive procedures. At the same time, works are being conducted on new-generation robotised, multi-functional surgical tools. These devices successfully passed the pre-clinical tests in vivo as well as the telesurgical simulation. The importance and originality of the Robin Heart project has resulted in the creation and development of a new field of science in Poland - surgical robotics. It is a chance for the introduction into clinical practice of modern procedures characterised by greater precision, safety, comfort and lesser traumatism.

Drawing on its long-established experience and potential, the Foundation also co-ordinates works on a family of mechanical heart assist devices. These include: an extracorporeal pulsation chamber for short-term heart support, used for several months in cases of acute circulatory failure; a partly implantable chamber supporting the chronically inefficient heart within a time-frame of several months to several years; a fully implantable heart prosthesis, ensuring support that is unlimited by time; and last but not least, a completely new element of the heart prostheses research in Poland: miniature centrifugal pumps, which may partially support the heart with continuous blood flow. Works on this project are being carried out under the long-term governmental programme: the Polish Artificial Heart, initiated by Professor Religa, with the participation of a dozen or so of Polish scientific and research institutes and several cardiac surgery centres. The Foundation carries out a significant part of the construction tasks within this programme, based on its substantial experience in extracorporeal heart support devices (in clinical use since 1996). They have been experimentally used in Buenos Aires and in six Polish cardiac surgery centres in the treatment of over 230 patients, supporting the hearts of patients waiting for transplantation or facilitating the regeneration of the defective organ. The oldest patient was 74, whilst the youngest, 12 years old.

The tasks realised so far within the Polish Artificial Heart programme, both in the research and the clinical part, stimulate further works on heart prostheses.

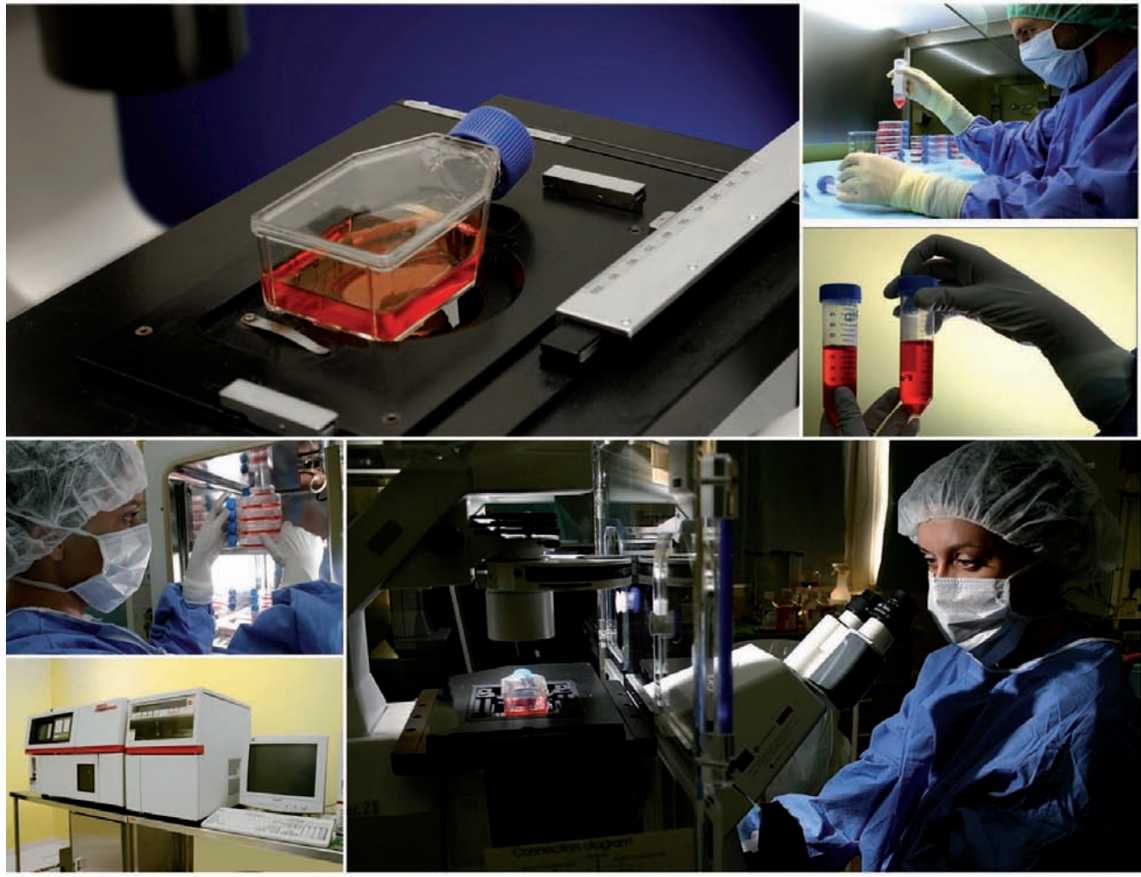
Accordingly, in response to the constantly growing need to treat ever younger patients, the RELIGA-PED project was born in 2008, with the objective to create a heart support system for children aged 8 to 12. Successful results of the clinical trials planned for 2012 would enable the creation of the first domestic treatment alternative in children with acute circulatory failure, perhaps giving rise to a new family of paediatric heart prostheses.

All these scientific, research, educational and training achievements of the Foundation would not have been possible without the donations, scientific research grants, subsidies, EU aid programmes, but first and foremost without the understanding and good will of the sponsors. That is why, to expresses gratitude to its benefactors, every year the Foundation organises the “Heart for heart” gala concert, inviting such distinguished artistes as Placido Domingo, José Carreras, Montserrat Caballé, June Anderson, Michel Legrand, Chris de Burgh and many others. The XX “Heart for heart” concert in 2011 will be the icing on the jubilee cake in celebrating the Foundation's 20th anniversary. Since its creation, the Foundation has consistently focused on innovative methods of heart treatment. The Foundation's legal form, the original multi-entity status, its management, significant scientific and research experience as well as its achievements in the commercialisation of technology, have all caused that it is a prestigious and internationally recognised institution. With this valuable capital and potential, we intend to continue Professor Zbigniew Religa's work.

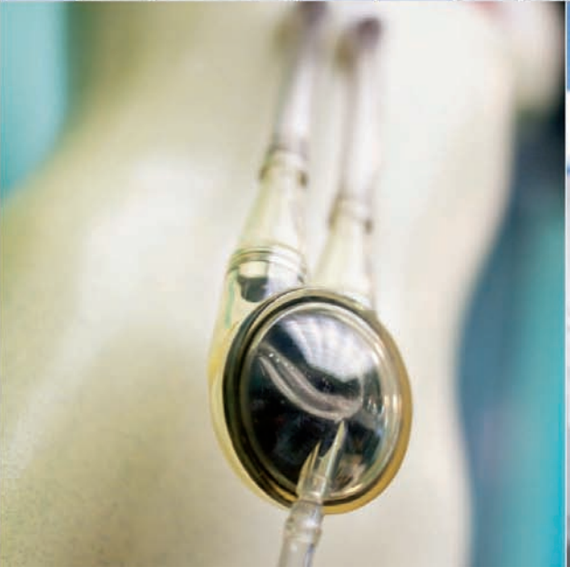
Dr Jan Sarna
Director General

Foundation of Cardiac Surgery Development 1995 & 2005 →

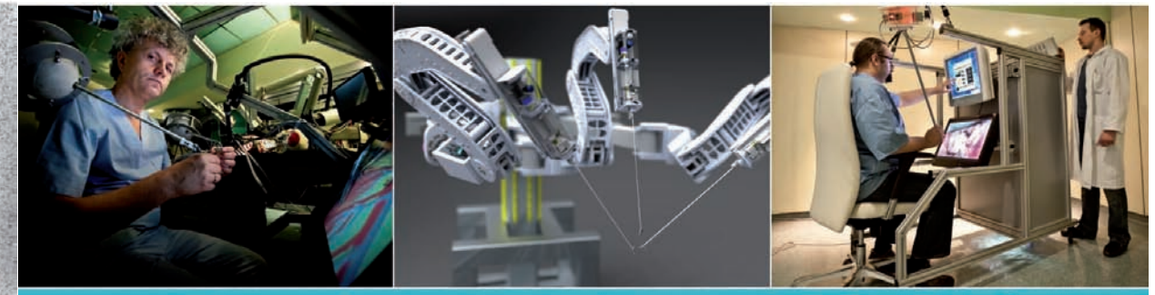
The Biological Valve Laboratory of Heart Prostheses Institute Foundation of Cardiac Surgery Development



The Bioengineering Laboratory of Heart Prostheses Institute Foundation of Cardiac Surgery Development



The Artificial Heart Laboratory of Heart Prostheses Institute Foundation of Cardiac Surgery Development



The Biocybernetics Laboratory of Heart Prostheses Institute Foundation of Cardiac Surgery Development

The Polish artificial heart – two perspectives, 20 years later.

By Zbigniew Nawrat

1. An objective history

The history of each innovation has its own heroes: engineers, physicians and … patients.

"Artificial organs contain both clinically established and emerging technologies: dialysis has proven its life-saving capabilities in hundreds of thousands of people for decades; cardiac assist devices have made tremendous clinical progress in recent years, with the 2-year survival rate increasing from 30% in 2003 to 70% today! [Shima 2011].

The rivalry between artificial heart and heart transplantation in the last decades has been fascinating: the first implantation of mechanical heart support in 1966, the artificial heart in 1969 but the first heart transplantation (HT) in 1967 and ... the first challenging problems. A triumphant return in 1982, with the artificial heart implanted as the target organ and ... success or defeat? Then, in the 80s, the introduction of cyclosporine: a medicine which prevents rejection of a transplanted organ, thereby increasing the chances of patients after HT. And then in 1985, the concept changed – the artificial heart came to use as a bridge-to-transplant. It was only 20 years later, that the idea of using the artificial heart as the target organ for patients threatened by sudden death but who could not undergo HT, revived. This procedure as well as the artificial heart were approved by the FDA in 2006.

But behind the history of these innovations, there were real people, with their ambitions and the fight on the edge of human possibilities and eternal natural laws. For example, although it was Cooley who performed the first artificial heart implant in a dying heart surgery patient, the artificial heart used was DeBakey's design and Cooley had used it without permission. Experimental animals also have their place in the history of artificial organs. There was an attempt to develop an atomic artificial heart and a great return to using turbine pumps. These pumps produce continuous flow, so the patient has no detectable pulse. Probably only de Bakey believed that it would be possible to design a pump, where the rotor turns at more than 10 000 rpm without blood degradation. He was eventually able to realise his dream with the help of ….one of his heart transplant patients, a NASA engineer. The timeline below shows major advances in world cardiac devices, together with Polish milestones.

WORLD MILESTONES IN CARDIAC DEVICES & Polish history

1812 A French physician, Julien-Jean Cesar Le Gallois, proposed the idea of artificial circulation.

1934 Dr. Michael DeBakey invented the DeBakey pump (peristaltic).

1937 An artificial heart designed by a Soviet scientist, W.P. Demichow, was first successfully applied in a dog for 5.5 hours.

1952 Charles Hufnagel sewed an artificial valve into a patient's aorta.

1953 Dr. John H. Gibbon, Jr., Jefferson Medical College Hospital, Philadelphia, performed the first successful application of extracorporeal circulation in a human, an 18-year-old female with an atrial septal defect.

1957 Dr. Willem Kolff and Dr. Tetsuzo Akutzus at the Cleveland Clinic implanted the first artificial heart in a dog. The animal survived for 90 minutes.

1960 Dr. Albert Starr, a surgeon from Oregon developed the Starr- Edwards heart valve: one of the most successful heart valves produced until the late 1970s.

1966 The first implantation of the de Bakey& Liotta mechanical heart support pumps.

1967 Christian Barnard performed the first heart transplant.

1968 Adrian Kantrowitz et. al., the first clinical trial in a man with intra-aortic balloon pumping.

1969 Denton Cooley, Texas Heart Institute, Houston, Texas, implanted a total artificial heart designed by Domingo Liotta. For 64 hours, the device served as a "bridge" for cardiac transplantation until a donor heart was found. The cardiac transplant functioned for an additional 32 hours until the patient died of pneumonia.

1982 Wiliam de Vries (USA, Utah) implanted the Jarvik 7 artificial heart designed by R. Jarvik, in co-operation with W. Kolff**.** The patient, Barney Clark, lived 112 days. The Jarvik TAH (pneumatically driven pump) was designed using a flexible four-layer diaphragm and a structural design that fits into the human chest. This design was a larger 100cc version of today's CardioWest TAH-t, which is 70cc.

1984 Loma Linda Medical Center, a baby girl's native heart was explanted and replaced with a baboon heart. She survived for 3 weeks.

1984 The first human implant and successful bridge-to-transplant with the Novacor® LVAS.

1985 At the University of Arizona, Dr. Jack Copeland successfully implanted TAH as a bridge-to-transplant.

1985 A successful heart transplantation in Zabrze (Religa).

1986 Implantation of BRNO LVAD (Z. Religa with the Vascu team from Brno).

1986 Introduction of the first atherectomy devices that remove material from the vessel wall.

1987 The first use of coronary stents (by 1997, over one million angioplasties had been performed world-wide).

1987 Implantation of the BRNO TAH VII (Z. Religa with the Vascu team from Brno).

1987 Implantation of the TAH POISK (Z. Religa with Shumakov from Moscow).

1989 The first xenotransplantation in the world; a pig heart was transplanted into a human by the Z. Religa team.

1990 The first LVAS patient discharged home with a Novacor® LVAS.

1991, 9th December signing of the notarial act establishing the Foundation of Cardiac Surgery Development; the first session of the Founding Board during which the Foundation Council and its Board were created.

1991 The first successful bridge-to-transplantation (Berlin Heart LVAD, Z. Religa).

1993, 5th March implantation of the Polish POLVAD (Z. Religa). The first Polish ventricular assist device was implanted in a patient at the Cardiac Surgery Faculty of Silesian Medical University.

1993, 4th September with a capital expenditure of $2 million, the official launch of the Institute of Research and Development of the FCSD, consisting of the Biological Heart Lab and the Artificial Heart Lab as well as experimental and clinical teams.

1994 The first FDA-cleared surgical assist robot (Automated Endoscopic System for optimal Positioning AESOP, manufactured by Computer Motion).

1994 FDA approval of the pneumatically-driven HeartMate LVAD (Thermo Cardiosystems, Inc.) for bridge-to-transplantation (the first pump with textured blood-contacting surfaces).

1994 HeartMate LVAS approved as a bridge-to-cardiac transplantation.

1996 REMATCH Trial (Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart failure, E. Rose principal investigator) initiated with HeartMate VE (Thoratec Corp.). The results published in 2002 showed mortality reduction of 50% at one year as compared to patients receiving optimal medical therapy.

1996, November A symposium organized at the FCSD on the state and perspectives for the development of an artificial heart, with the participation of famous specialists in this domain: Prof. Adrian Kantrowitz, University of WAYNE, Detroit (the constructor of the world's first devices for mechanical support of the heart muscle), Prof. Yukihiko Nose, Baylor College of Medicine, Houston (the Head of International Association of Artificial Organ - ISAO, Founder of the best research center of heart assist devices in the USA), Prof. Peer M. Portner, Stanford University (co-designer of the first, clinically used, implantable ventricular assist device NOVACOR), Prof. Kou Imachi, Institute of Medical Electronics, Tokyo University, Kunihiko Mabuchi, Shin - ichi Nitty, Japan, Klaus Affeld, Virchow - Klinikum Humboldt Univ. in Berlin, Helmut Reul - the Head of Biomechanic Faculty of Bioengineering Helmholtz Institute in Aachen and Heinrich Schima - the Head of Biomedical Research Center in Vienna.

1997, 19th -22nd Jan Prof. Sir Donald Ross from London, a pioneer in the development of biological valves, visited the FCSD.

1997, 29th Sept Prof. Rene Favaloro from Argentina - the creator of a CAD treatment method based on bypass grafting, and Alejandro Juarez Hernandez, the Head of the National Institute of Cardiology in Mexico visited the FCSD

1998 Simultaneous FDA approval of HeartMate VE (ThermoCardiosystems) and Novacor LVAS (Baxter Healthcare Corp), electrically-powered, wearable assist systems for Bridge-to-Transplantation

1998 The first clinical application of the next-generation continuous-flow assist devices. The DeBakey (Micromed Inc.) axial-flow pump was implanted by R. Hetzer, G. Noon and M. DeBakey.

1998 Carpentier & Loulmet performed the first in the world endoscopic operation of a single bypass graft between the left internal thoracic artery and the left anterior descending artery (LITA - LAD).

1998 The first operation inside the heart - mitral valve plasty and atrial septal defect closure (da Vinci).

1998 Mohr & Falk bypass surgery and mitral valve repair using the da Vinci robotic system.

1999 The first clinical application of a fully-implantable circulatory support system. The LionHeart LVAS was implanted in a 67-year-old male recipient by R. Koerfer and W. Pae.

1999 D. Boyd: the first totally endoscopic Zeus-based coronary artery bypass graft (E-CABG).

2000 The first implantation of Jarvik 2000 by physicians in Houston US; recruitment of the first patient for the clinical investigation of the Jarvik 2000 Heart.

2000 A multidisciplinary team began works on the creation of a Polish cardio-robot, the Robin Heart, at the FCSD in Zabrze (Z. Religa & Z. Nawrat).

2000, 19th Nov the first implementation of the Polish VAD in Argentina, the Clinic of Transplantology and Cardiac Surgery of Favaloro Institute in Buenos Aires.

2001 Dr. Laman Gray and Dr. Robert Dowling in Luisville (Kentucky, USA) implanted the first autonomous artificial heart - AbioCor (Abiomed, Inc, Danvers, Mass).

2001 The first trans-Atlantic telesurgery - Lindbergh operation – a surgeon from New York operated on a patient in Strasburg using the ZEUS system.

2001 **T**he first fully-implantable TAH Lion Heart (the Texas Heart Institute in Houston, Abiomed in Danvers, US) was used.

2001 The AbioCor totally implantable, electrically-powered TAH was implanted into a patient, Robert Tools, by L. Gray and R. Dowling (the clinical trial is ongoing).

2002 FDA approval of the HeartMate VE LVAD for permanent use (Thoratec Corp.).

2007 Start of the Polish Artificial Heart Project (long-term, governmental Artificial Heart Program), aiming to develop a full set of blood pumps assisting or replacing the heart, including a totally implantable artificial heart.

2009 The first animal experiments using one of the Robin Heart Family robots (cholecystectomy, elements of valve operation).

2009 The first in Poland implantation of the HeartWare blood pump (first - G. Religa, Warsaw, next - J. Pacholewicz, Zabrze).

2010 The first animal experiments using the new Robin Heart mc^2 robot (elements of bypass surgery on a pig).

Fig. 1. William Kolff in Kampen and in Gent (photo performed by author). In the laboratory of Willem Kolff, small ventricles with semi-elastic shell were worked out. They were made from Pellethane 80AE / 2363 (Dow Chemical, Japan) by the vacuum forming and glued by dielectric welding. Valves are made from Isoplast, covered by Pellethane and glued. Presented ventricle fulfils the dream of Kolff – to create easy in production, cheap ventricle for every one. W. Kolff sent the sets of ventricle to many centres in the world, in the aim of the realization experiments, to Poland as well. We carried out in Zabrze comparative in vitro investigations with these ventricles to detect the level of artificially inducted forms of thrombus.

The rapid development of biomedical engineering has created new possibilities of helping people with heart diseases. Over the last five decades, blood pumps

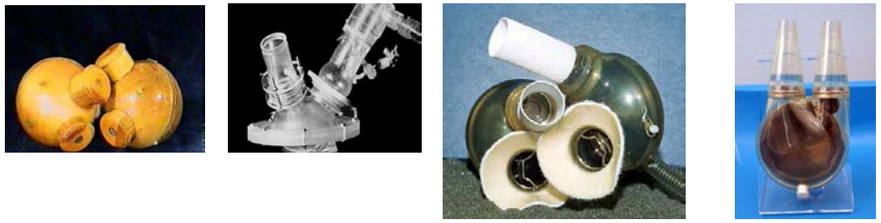
of various constructions have been introduced into clinical practice, with the aim to partially support or replace the heart during open heart surgery, over considerably long periods of time needed for heart recovery, or until transplantation. The new construction of the Total Artificial Heart and Ventricular Assist Devices (**TAH & VAD**) offers new hope for millions of heart patients whose life expectancy is greatly reduced.

A VAD is an extracorporeal pneumatic blood pump (connected to the Control Unit), invented to assist the failing left or right ventricle, or the whole heart, until recovery or replacement by means of transplantation. A TAH is designated for emergency replacement of the irreversibly damaged natural heart. Because the natural heart is completely removed, TAH must ensure the hemodynamic, regulatory and control function of the circulatory system.

In Europe, the most advanced mechanical heart support systems were developed in Aachen (MEDOS), Berlin (Berlin Heart) and Zabrze (Religa Heart).

The real starting point of our history was the first successful heart transplantation performed by Zbigniew Religa and his team in Zabrze in 1985, followed by setting up, a few years later, the Artificial Heart Lab at the Cardiac Surgery Department of the Silesian Medical Academy (now – Medical University of Silesia). In 1991, the Foundation of Cardiac Surgery Development was established, with a purpose of implementing state of the art methods of saving human life when the heart is threatened. Currently, we are conducting research and development work in our own Institute of Heart Prostheses (director Zbigniew Nawrat) which includes the Biological Heart Valve Lab (chief Jolanta Wszołek), the Artificial Heart Lab (chief Roman Kustosz), Biocybernetics Lab (chief Zbigniew Nawrat), and the Biotechnology Lab (chief Piotr Wilczek).

The pneumatically driven, membrane type Polish Ventricle Assist Devices **POLVAD** (U-shaped) and the Artificial Heart **POLTAH** (spherical type of geometry) were developed in Zabrze, Poland [Fig 2]. POLTAH has since changed its name to Religa Heart. The first POLVAD was implanted in 1993. To date, over 240 clinical procedures of VAD implantation have been carried out. In a selected group of patients, long-term mechanical circulatory assistance may lead to myocardial regeneration or may serve as a bridge to transplantation. In his article, Pacholewicz [2009] presented the case of a 16-year-old boy suffering from heart failure due to ventricular noncompaction. Because of a significant clinical worsening of the patient's condition, a biventricular assist device (BIVAD) was implanted using the POLCAS-RELIGA system. Over a period of almost one year, BIVAD treatment ensured the stabilization of hemodynamic parameters and a reduction of pulmonary resistance. After 358 days of assisted circulation, a successful heart transplant was performed.



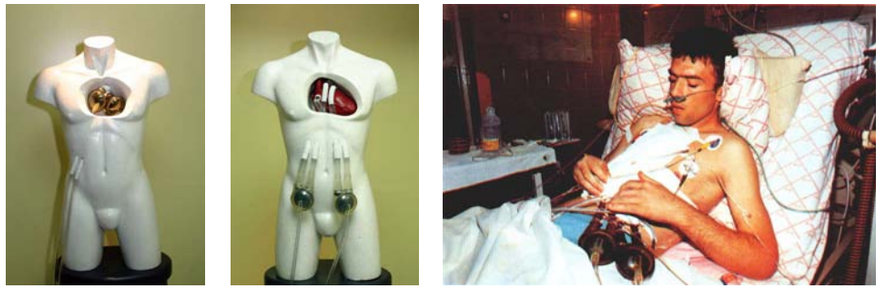
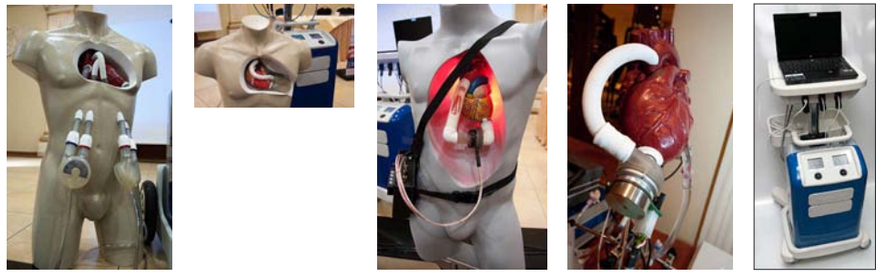


Fig.2. The steps of POLTAH design process (model after cadaver study, transparent model for laser visualization investigation, first polyurethane model for start animal experiments). Non-symmetrical shape of POLVAD proposed & designed by author create very good flow internal flow condition. The first successful POLVAD implantation (T.G. 1993). Till now about 240 POLVAD clinical implantation, over 1 year the longest time successful application. Pneumatically driven artificial heart (Poltah) and ventricular assist device (Polvad).



POLVAD-EXT **POLVAD-IMPL** **POLVAD-ROT** **POLVAD-ROT2** **Control unit 501 N 006**

Fig. 3. The national strategic project "Polish Artificial Heart" No: 2/0-PW/PO1-PBZ-MNiSW/2007 results. The Polish Mechanical Heart Support Systems.

Stages of the preparation of a new design and prototype:

1. Analysis of anatomical and physiological data;
2. Designing with computer design software;
3. Computer-simulated tests;
4. In vitro tests of the prototype using a specialized test stand (Mock Circulatory Investigation, Laser Flow Visualisation LFV, Laser Doppler Anemometer LDA made by Zbigniew Małota);
5. In vitro tests with natural tissue, blood etc.;
6. In vivo tests on animals;
7. Clinical application.

At the same time, clinical trials with another type of blood pumps (from Brno and Moscow) were prepared (readiness of the clinical and technical team).

The Berlin Heart VAD was used in Poland by Z. Religa's team in the first successful mechanical cardiac support as a bridge-to-transplantation. I was responsible for the technical part of the procedure (controlling) during this intervention (May 1991) and for the next two weeks I supervised the work of the ventricles driven by the Polish drive unit JSN 301. The patient, Tomasz Gruszczyński was awaiting heart transplantation. After 11 days of support, a heart transplant was performed and the patient returned home. Unfortunately, the heart was rejected after two years and the patient needed another new heart. In the meantime, together with a chemist, B. Stolarzewicz, I designed a new Polish ventricular assist pump, named POLVAD. As it happened, the first patient to use POLVAD was the same patient, T. Gruszczyński. That period was the most important test in my entire life. Fortunately, the POLVAD worked very well and after 21 days, a heart transplant was performed. Nevertheless, the patient died during surgery, his second heart transplantation.

A journalist summed up the situation by writing: "A technician's success, a physician's failure".

2. THE PATIENT – a subjective history

Twenty years have passed since the first success of Prof. Religa's team in the field of mechanical circulatory assist devices. The history of each innovation has its own heroes: engineers, physicians and … patients.

Tomasz Gruszczyński, a patient with irreversible heart failure from Bielawy (PL), was just as much a hero as the members of the medical and technical team. Although transplantations were already carried out in Zabrze, the heart assist devices used did not guarantee patient recovery. The longest functioning was a Russian artificial heart, which lasted 10 days. The best patient condition was achieved with a Czech heart, in a patient operated on by Religa's team in Katowice. Although three days later a heart donor was found, sadly, the patient died immediately after transplantation.

In Tomek's case, however, for the first time, the Berlin Heart assist devices were used. When I saw Tomek, he was writhing in his bed unable to breathe comfortably. The heart can be successfully transplanted only to a patient whose all other organs are in good condition. There were concerns as to whether Tomek would make it to the transplantation as diuresis problems began. The risk was growing but there was no donor. Finally, it was decided to use a pump.

Although we had been working on a Polish pump for three years, at that time, we were still not ready. Prof. Religa, who knew the good results of the Berlin Heart assist system, did everything humanly possible to help the 16-year-old. The Ministry agreed to purchase the assist device at an astronomical for those times price of 75,000 DM each (more or less the amount we were given as our first 3-year scientific grant). On board of a military aircraft in an "emergency" mode, Romuald Cichoń flew to Berlin to collect the devices for their immediate implantation in Zabrze. The pneumatic pumps would be fed and controlled by the already functioning Polish artificial heart drive system.

Everything was going perfectly. Tomek's recovery was startling. He quickly became everybody's favourite on the ward. I did not leave the hospital for days. An idea was percolating in my head. Finally, we convinced ourselves and everybody else that artificial pumps can replace the natural heart also in Poland. In the meantime, however, the patient's good condition presented an all-new problem. How to ensure psychological wellbeing for a person connected to a machine…. indefinitely? As I was always by Tomek's side, I began to feel responsible for his free time. I provided him with a radio and a TV set. Video technology was on the rise and everybody in the hospital knew to bring Tomek new films. We also spent many hours talking. Tomek believed that nothing wrong could happen to him. He was much braver than all of us put together. I still remember his unemployed parents bringing him his favourite "Michałki" chocolates.

We were looking for a donor and Tomek became directly involved in the search. With the help of the media, we encouraged the nation not to waste natural organs. "Never send to know for whom the …. heart tolls; It tolls for thee". And so we were waiting for the heart. With relative tranquillity, as we had the efficient mechanical assist device on our side. When finally I was told that there was a donor – the emotions reached a boiling point. I remember my surprise at the simplicity of Tomek's reaction - he was just looking forward to leaving the hospital and making a full recovery.

After 10 days of mechanical heart assist, on the night of 28th May 1991, Prof. Religa's team performed cardiac transplantation. Together with his young team, he operated as if this type of surgery was a routine treatment. There was an absolute silence in the room as he sutured the heart. Stimulation with electrodes and… the long-awaited divine rhythm of life finally filled the room.

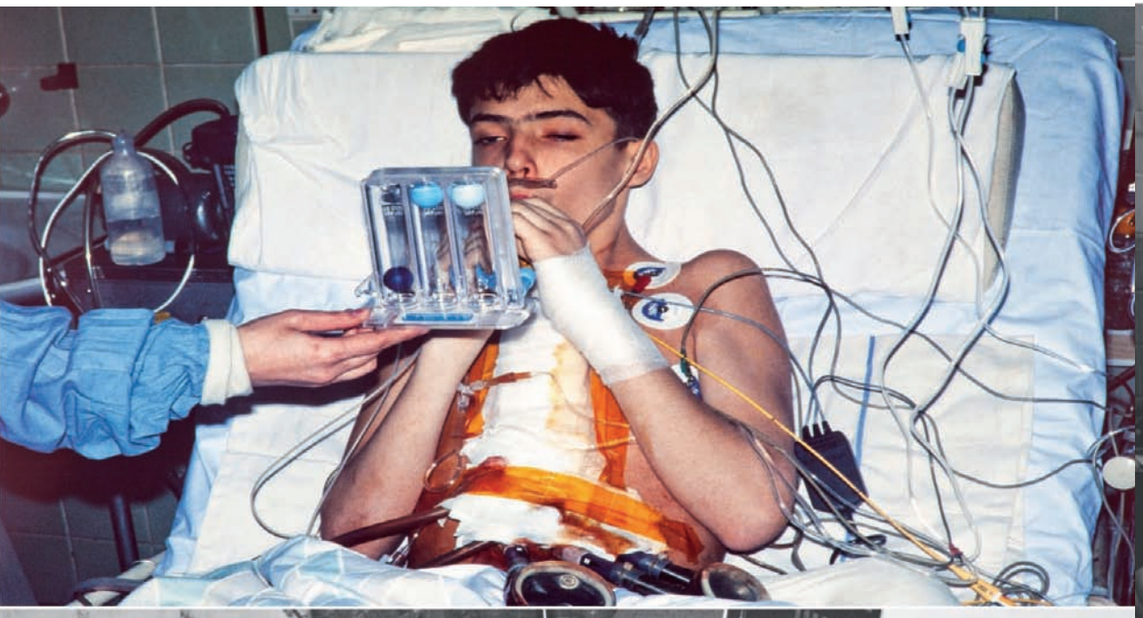
After the transplantation, Tomek often sneaked out of hospital so as to be able to enjoy the outdoors, even if only for a moment. Whilst it was true that the pumps had given him a chance for surviving, they had also taken away his freedom. Freedom that only heart transplantation was able to bring back. Tomek returned to his friends and family in Bielawa, where he became a local hero. The handsome and interesting young boy was slowly becoming a man. He was a DJ, had a beautiful girlfriend, and even became accidentally involved in a criminal plot. I still willingly show a film with him jumping down 7 m head-first into a pond. We gave him the chance for a new Life and he lived it to the full. Sadly, after two years, the heart transplant was rejected. To this day, the exact cause remains unclear. With Tomek's courageous attitude to life, discontinued pharmacotherapy was hypothesised, though I refuse to believe this.

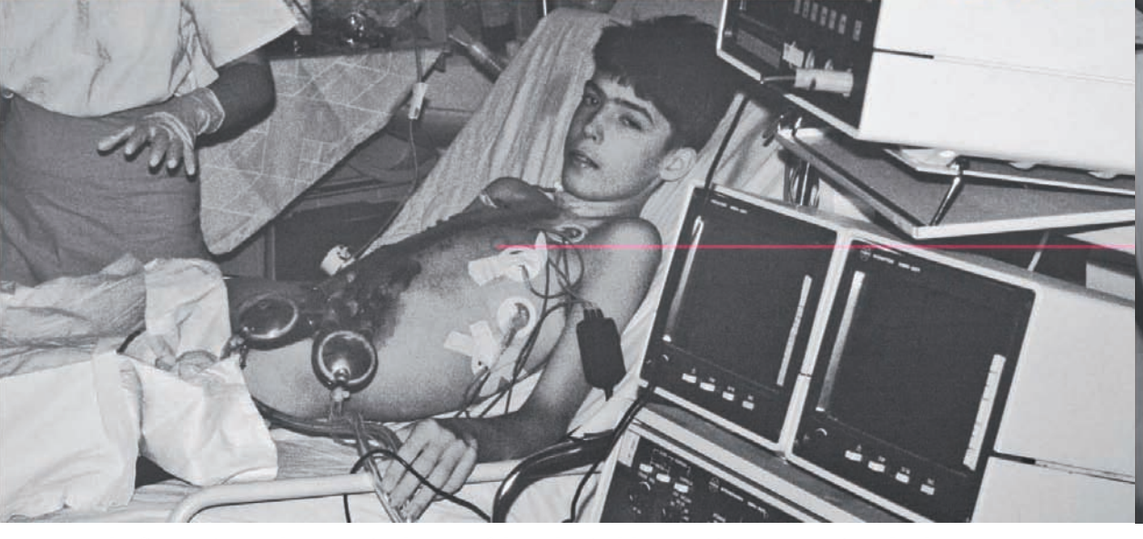
For me, the 1991 surgery was a major event: I realised that I really was the doctor's partner. Inspired by the German devices, I designed the 80 ml output POLVAD – similar to the Berlin Heart. However, my research experience, especially in laser flow visualisation, suggested a new solution for the shape of the ventricular cup. POLVAD is a unique ventricle of an asymmetrical geometry, optimal for blood flow. Several years later, I showed it to R. Jarvik and W. Kolff.

In the meantime, my old friend, Tomek, returned to the clinic in Zabrze due to transplant rejection. At that time, our POLVAD ventricle was ready. We moved into action without hesitation. Tomek had no doubts that he would make it again. Not only would he become the first patient whose heart was mechanically assisted twice but also the first patient to undergo a second successful heart transplantation! I was eager to see our blood pumps put to the test. Two years had elapsed since my first draught. I wonder if so quick (and cheap) introduction of a new blood pump will ever be possible again. After several days from surgery, the ventricular assist devices become synchronised with the heart's function, based on the ECG. However, in Tomek's case, due to a leaky valve of the transplanted heart, standard procedures were not an option. Sadly, Tomek did not survive the second transplantation. He died at 1.40 am on 20th June 1993 during surgery. The fourth operation within the same site proved insurmountable.

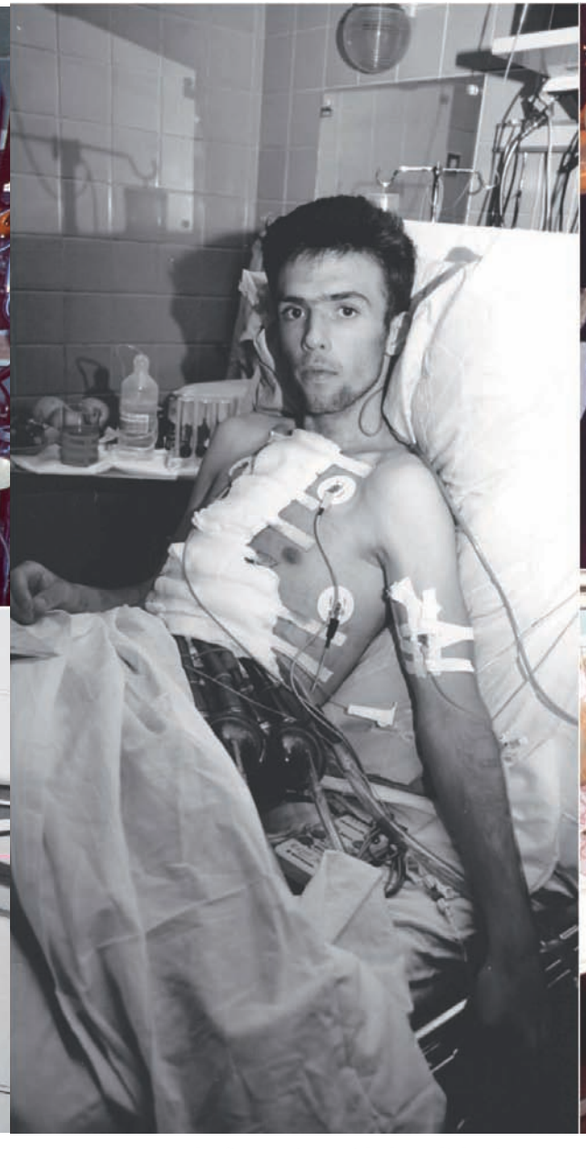
Our role as engineers and doctors is to make the patients recover completely and forget about the past disease. The newspapers wrote "an engineer's success, a doctor's failure". That is a fallacy. There is only the patient's success or a failure of us all.

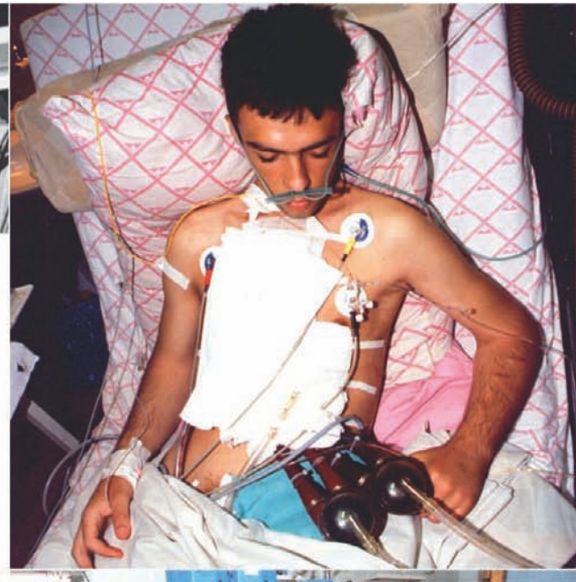
PS. As I am writing these words, there are three patients with POLVAD ventricles and one patient with the Berlin Heart paediatric ventricle at the Silesian Centre for Heart Diseases in Zabrze. Another four patients are at home with an implanted HeartWare pump. Although the Father of Polish transplantology and the artificial heart, Prof. Zbigniew Religa, is no longer with us, more and more patients receive hope for a new life with the POLVAD assist ventricles, now called *Religa Heart*.

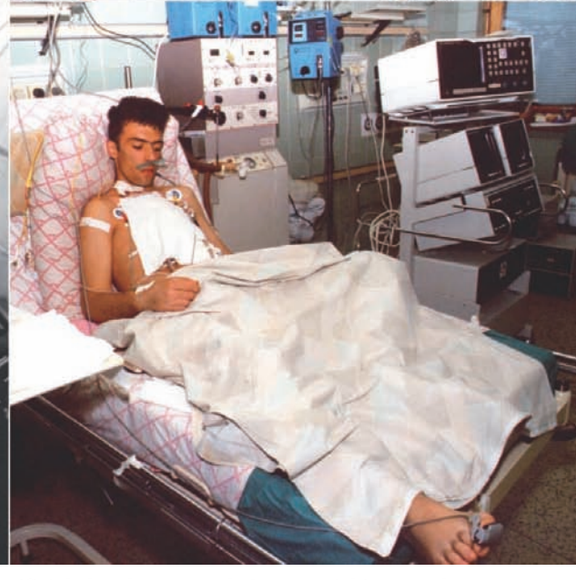




The first patient to have received successful mechanical heart assist treatment in Poland. In May 1991, after 11 days of mechanical assist with the Berlin Heart, Tomek Gruszczyński underwent heart transplant surgery carried out by Professor Religa's team.







The first patient with the Polish POLVAD artificial chambers. Tomek Gruszczyński was re-hospitalised due to heart rejection. Thanks to the POLVAD chambers, he survived 21 days waiting for the next transplant surgery. Sadly, he died during the operation.

Explanted heart prostheses as a source of knowledge.

Report. From the clinic to the laboratory to improve heart prostheses and biomaterials.

Comprehensive evaluation of explanted cardiovascular prostheses within the European network of laboratories. A short summary.

Z. Nawrat

Every year, hundreds of thousands of heart valve and blood vessel prostheses, pacemakers and blood pumps are implanted in order to improve the efficiency of the circulatory system and to restore health in approximately one million patients. Device and material implantation is becoming an increasingly common element of the treatment process. Dysfunctions and biodegradation are the main causes of reoperation and prosthesis replacement as well as an inspiration for the search for better materials and technological solutions. The absence of an efficient research system on explanted prostheses affects both the doctors, who wish to know the causes of a given patient's condition and the estimated development of device failure with a view to optimise the planned treatment, and the producers and constructors, who contribute to better treatment results through improved technologies and technical solutions.

The final report shows that: the plans for organising a network of laboratories and clinics have been realised; a bank of explanted materials (currently encompassing over 140 heart valve, vessel and stent prostheses) has been created; research procedures have been drawn up based on the substantial material research instrumentarium; methods of physical and computer modelling have been introduced into the analysis of pathologies present in the collected valve prostheses; new research stations have been designed and constructed; the collected prostheses and materials have undergone extensive research; and a database with an internet site has been created to serve as a forum for the exchange of research information. A book, a collection of articles by the project partners, is being prepared.

The majority of the research dealt with heart valve prostheses due to their prevalence of use, access to explants and the cognitive value (various materials, work under load in the blood, varying implantation time). Heart valve implantations are a standard in cardiac surgery in cases of advanced dysfunction

of valve cusps and the perivalvular apparatus. Despite the increasingly modern methods of both biological and artificial valve preservation, in a number of patients, long-term complications occur. The character of the pathological changes is partly connected with the type of the implanted valve. In the case of bioprostheses, they involve: valve degeneration with calcification, infective endocarditis, thrombotic lesions within the valvular apparatus, cusp rupture, and hyperplasia of the tissue surrounding the valvular apparatus. With regard to mechanical valves, the most frequent issues are: loose sutures, hyperplasia of the tissue surrounding the valvular apparatus and infectious inflammation. In both types of the implanted valves, hyperplasia of the implant-surrounding tissue is observed.

From the morphological point of view, three working hypotheses as to the potential causes of the pathological changes can be assumed:

- excessive resection (damage) of the perivalvular tissue during preparation of the valve implantation site;
- activation of a chronic inflammatory reaction within the valvular apparatus before implantation;
- presence of intense mechanical stress (suture kind and type) within the implantation site.

In all the examined samples of explanted valves, signs of a persistent chronic inflammatory reaction were observed, the exponent of which was the presence of excited CD3 positive lymphocytes and activated macrophages. In the majority of the examined samples, an elevated number of mast cells and endothelial VEGF (vascular growth factor) expression were seen. Cell-rich connective tissue with numerous fibroblasts and fibrocytes was predominant. There was a correlation between the presence of activated macrophages and mast cells and the increase in VEGF expression. The majority of the implanted valves displayed regressive/degenerative changes in endocardial connective tissue, especially fibrosis and hyalinisation. Presumably, this process is connected with the increased mechanical properties (resistance) of the endocardium. In most cases, including short-term valve implantation, focal endocardial calcifications were present. Interestingly, perivalvular endocardial endothelium became renewed. In some cases, where examinations encompassed material with well-preserved surgical thread, it was possible to see the ingrowing of the thread into connective tissue and the creation of collagen between filaments. The resorptive reaction around the thread was insignificant. However, in one case of resorptive reaction, the creation of a scavenger giant-cell (around-foreign-body type) with lymphocyte fusion was noticed. These cells are created by the union of scavenger cells in the process of foreign body removal/resorption; the

multitude of nuclei may be of some significance to their biology and longevity. Biological valve cusps showed a varying level of degenerative changes. In some cases, the structure of the cusp was homogeneous, with blurred details of the stem tissue, whilst the surface was covered with pseudoendothelium containing macrophages, lymphocytes and other mesenchymal cells among the loose fibrins. Some biological valve cusps revealed numerous fissures between the collagen bundles. The pathological proliferation of connective tissue was accompanied by a chronic active inflammatory reaction within the perivalvular tissue. Further research is warranted to show why in some patients the acute perioperative inflammatory process transforms into a chronic process with pathological proliferation of connective tissue.

Macrophages, fibroblasts (fibrocytes) and mast cells constituted the prevalent cellular element in the examined samples. The samples were characterised by a varying level of tissue remodelling: from cell-rich with poorly organised extracellular matrix to highly organised changes and hyalinisation. Clinical information on the observed haemodynamic effects of valve failure was verified experimentally with the use of testers – flow simulators.

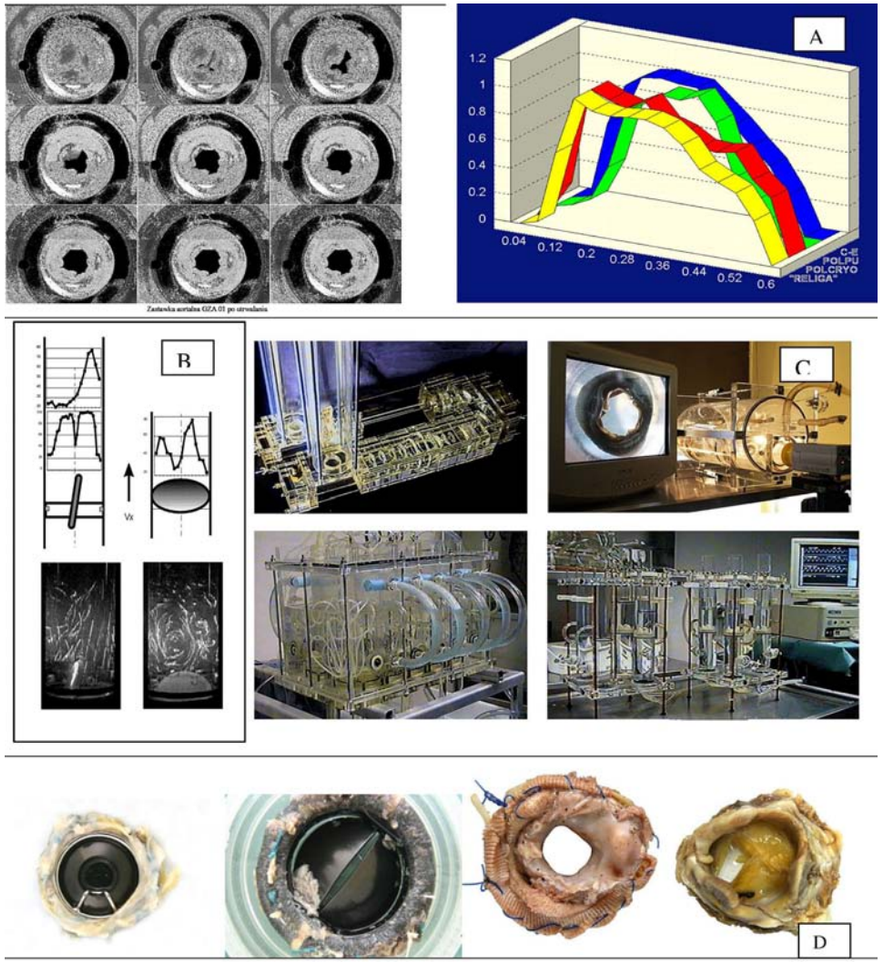
What makes our project original, is the implementation of computer and physical modelling techniques in evaluating haemodynamic and resistance effects associated with various heart valve dysfunctions and surgical errors. The available models include valves with cusp perforation, cusp calcification, paravalvular leak and a homograft implantation model with twisting. Depending on its location and extent, cusp perforation causes varying degrees of back-leak, but also decreases the forward blood flow resistance. The change of mechanical properties, e.g. as a result of calcification, leads to disorders of blood flow through the valve and, subsequently, to changes in the haemodynamic parameters of the entire circulatory system. It is very frequently connected with stress distribution over the valve cusps. In the proposed model, the most advanced heart model of this type in Poland, we can observe a visible drop in coronary vessel flow. Research with physical models involved valves (natural porcine aortic homograft) and was carried out under precisely determined VAD conditions and the system's physical properties: resistance, susceptibility and volume. Depending on the type of deformation introduced (surgical error model), changes in flow intensity (flow profile) and regurgitation were observed. The obtained information may be of clinical importance and may influence the creation of methods to be introduced during valve implantation in selected cases or during postoperative treatment with a view to prevent unfavourable phenomena.

The www.ImlantExpert.eu website facilitates the presentation of achievements of a multidisciplinary research team and the project's network of laboratories and clinics. We hope that it will not only serve the propagation of the idea of research on explanted materials, but will also become a living forum for the exchange of experience and a source of continuous inspiration for technological progress in the field of implants, prostheses and the relevant treatment methods.

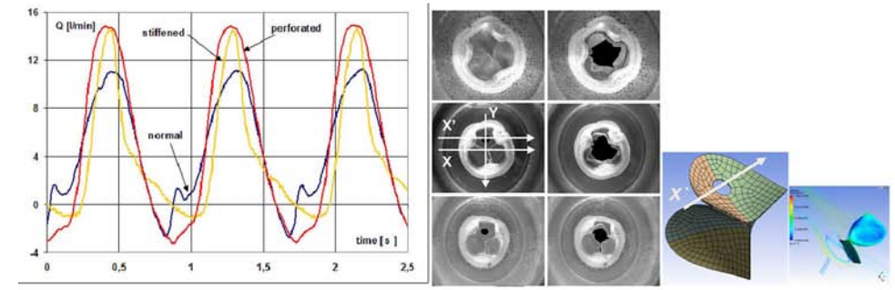
On completing the project, the Foundation of Cardiac Surgery Development took over the financing of activities involving explant research, propagation of the idea of advances in treatment methods and the creation of new materials and prostheses based on explant research analysis. Hence, the process that commenced within the European COST 537 project and developed as a Polish special project, shall be continued.

If the provisions of the European project become translated into new legal regulations and requirements concerning the unification of research on explanted prostheses, national clinical and research centres will be able to benefit from the organised infrastructure and the experience gained. The completed project is a continuation of the European COST 537: Core Laboratories for the Improvement of Medical Devices In Clinical Practice from the Failure of the Prostheses Analysis (FEPA) project, the aim of which was the drawing up of research procedures, the creation of a network of laboratories and a database encompassing research results in several groups of commonly used prostheses. In compliance with the project requirements, each country entering the network had to self-finance its research and develop a uniform and effective national system in accordance with the agreed homogeneous European procedures. What is worth emphasising, although the realisation of this task has already been fulfilled within the Polish project, all procedures will continue within the organised network of laboratories and clinics.

The conducted research, partly published and presented (partly still being edited), testifies to the significance of this venture and its results to clinicians and engineers. Research into dysfunctional prostheses and the modelling of destruction processes may facilitate the prediction of treatment effects and the optimal selection of prosthesis for each patient. Finding the right answers will also entail positive economic consequences in long-term patient care.

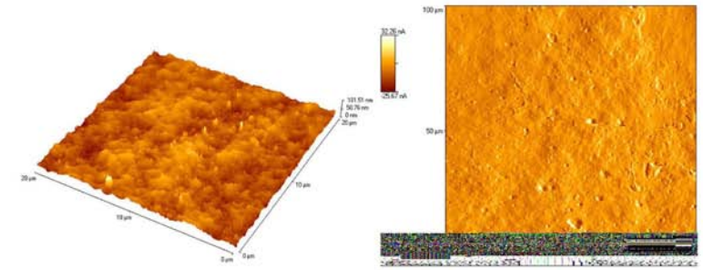


Valve tests and the valve tester designed and introduced at the Institute of Heart Prostheses of the FCSD. The valve opening area test and one-cycle comparison of the biological Religa prototype, Polcryo, the synthetic Polpu prototype and the Carpentier Edwards bioprothesis [A]. The results of laser visualization and laser anemometer test of the Polish prototype of the valvular disc [B]. Some of the testing apparatus used at our Laboratories [C]. A single-disc and a double-disc mechanical valve, and two bioprostheses from the Explanted Prostheses Bank.

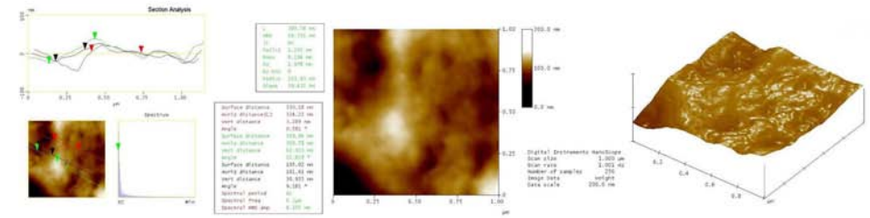


Physical and computer modelling of valvular heart disease - a comparison of flow profiles in: a normal, perforated and a calcified valve.

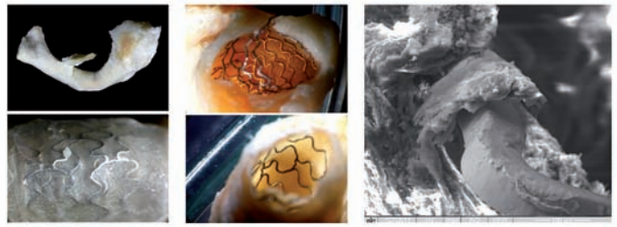
A clinical sampling kit and sample valves: biological and mechanic.



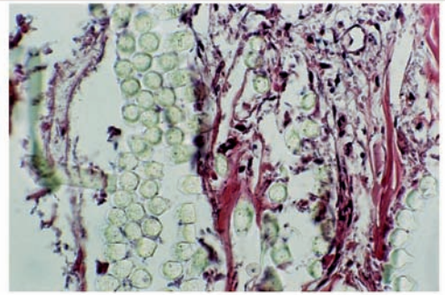
A sample examination – an atomic force microscope image of the valvular disc.



A sample examination – an atomic force microscope image of vascular prostheses.



Sample examinations of vascular stents.



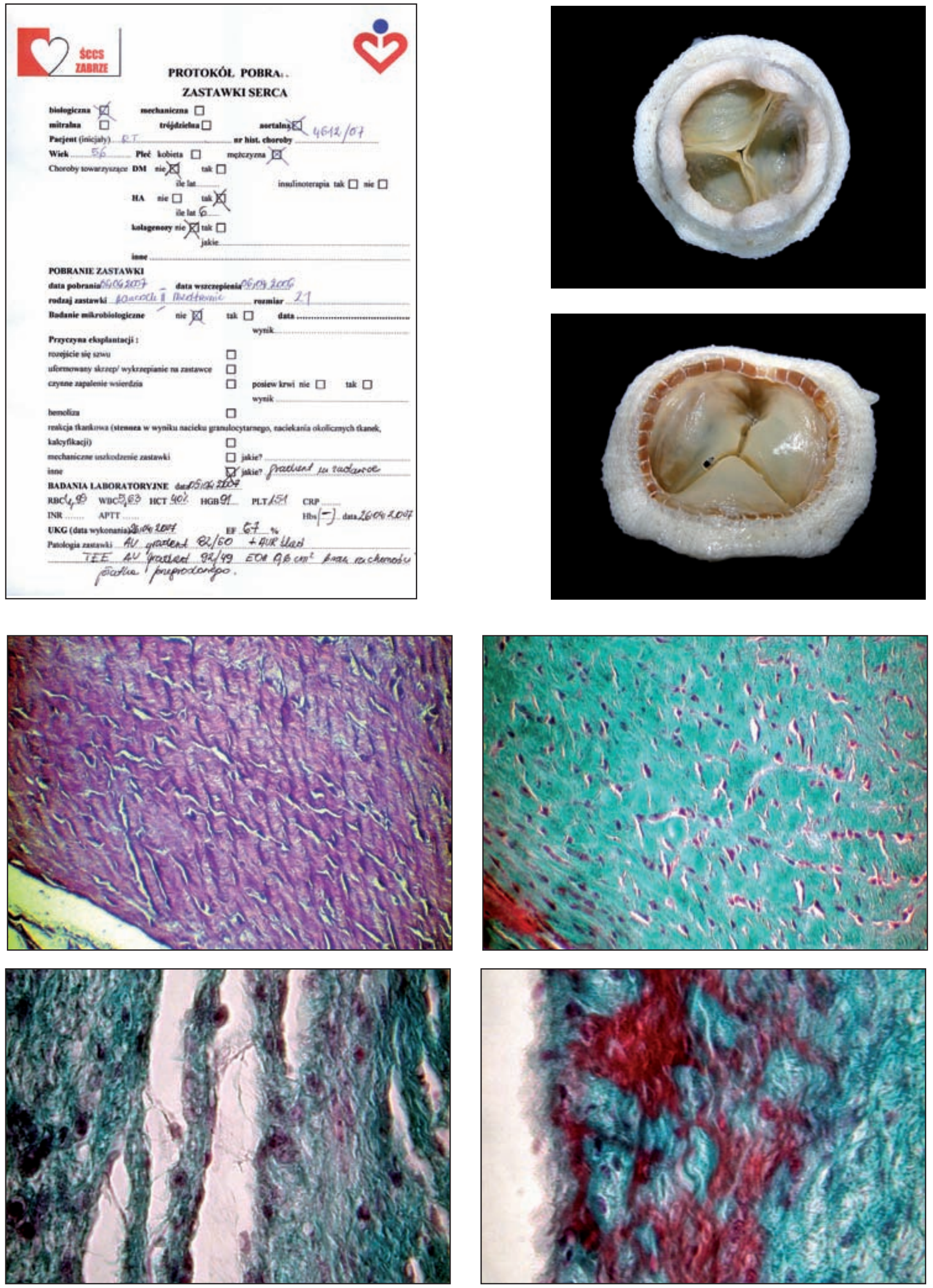
A sample examination – the histopathological image of the thread used to suture the prosthesis into the heart

The achievements of our Polish team were recognized by the organisers of the European COST 537 project ("*Core Laboratories for the Improvement of Medical Devices in Clinical Practice from the Failure of the Explanted Prostheses Analysis (FEPA)*") who entrusted us with the organisation of the spring school workshops in Zabrze from 15th to 17th May 2008, closing the European WG2 Training School project. During the training sessions, lectures and laboratory workshops, we presented to a group of young scientists and students interested in developing a network of laboratories in their countries, the scientific foundations, procedures and practical aspects of the system on the example of the Polish group focused around the Foundation for Cardiac Surgery Development.

Patient case study

Joanna Śliwka
Silesian Centre for Heart Diseases
Zabrze

In April 2006, due to post-inflammatory valve degeneration, a 56-year-old man with no significant health history, had a 21mm biological Hancock II Medtronic® valve implanted in the aortic position. One year later, the patient was referred for cardiosurgical consultation due to suspected valve dysfunction. The patient reported no stenocardial ailments, NYHA class II, healed wound and a stable sternum. Blood test results were within normal ranges. Transthoracic ultrasonocardiographic examination revealed a dysfunction of the biological prosthesis, namely stenosis with aortic valve area of 0.8cm2 and pressure gradient max 82 mmHg (the norm for this valve <20mmHg) and mean 50mmHg, no septal cusp mobility, with very good overall contractility (EF 67%). The examination also revealed a significant widening of the ascending aorta. Valvular dysfunction was confirmed by transesophageal ultrasonocardiographic examination. The patient qualified for reoperation - replacement of the biological prosthesis and the ascending aorta. The procedure was performed with extracorporeal circulation (330min, with aortic clamping time 263 min and circulatory arrest time 40min) and a 23mm Medtronic ADVANTAGE SUPRA MECHANIC valve was implanted in the aortic position. The biological prosthesis was assessed intraoperatively, revealing stiffening of the noncoronary cusp. The valve was transferred to the Foundation for the Development of Cardiac Surgery for further examination. The postoperative course was uncomplicated. A valve tester revealed slightly impaired mobility of one of the cusps with a non-physiological place of bending, where a small perforation occurred. Pressure measurements did not confirm the initially diagnosed prosthesis stenosis. It is hypothesised that the increasing subaortic stenosis (probably resulting from prolonged hypertensive disease and myocardial hypertrophy, and especially hypertrophy of the interventricular septum) caused the high pressure gradient, which imitated prosthesis stenosis.



The valve protocol and pictures (small leaflet perforation). Histology of explanted valve(performed by J.Nożyński) showed extensive endocardial fibrosis, confirmed by connective tissue stain, Masson trichrome collagen stains green. Valve leaflets histology showed the presence of delamination tissues, located between the spongyform and compact layers, whereas at the venricular space the acidophilic deposits were found (intensely stained red on last picture). No inflammatory infiltrations were found. Thus, the leaflet incompetence resulted form structural, and probably humoral alterations.

Valvular bioprostheses explants studies in Turin.

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In a previous paper, published in 2008[1], we presented our long experience in the study of cardiovascular implantable devices, gained in the Biomaterials Laboratory of the Veterinary Morphophysiology Department, University of Turin. On the strenght of the gathered knowledge, we proposed detailed guidelines for the morphologic study of explanted valvular bioprostheses. The guidelines facilitate the detection and description of all the possible aspects of valvular degradation and dysfunction as part of routine laboratory work. The aim of the procedure is to point out macro and microscopical observations and morphometric evaluations. The results are then collected in a report, which enables scientists aware of the industrial, surgical and pathological implications of prosthetic valves to draw conclusions. The report can also give useful feedback for both manufacturers and surgeons.

As the University promotes the founding of spin-off companies, in March 2008, a group of researchers working in the Biomaterials Laboratory decided to establish a company named Life and Device in order to apply the acquired competences and to direct our young graduates into qualified work. Life and Device is a service provider, specialized in studies on cardiovascular implantable prostheses, artificial organs and, more widely, biological interfaces. The company is a specialist in anatomical, histochemical, immunohistochemical and morphometric analyses; each analysis is carefully adapted according to the requested study and device characteristics.
Life and Device offers a wide range of services:

1. Assists manufacturers of biological and synthetic prostheses in compliance and safety checks, both in pre-clinical and clinical phases.
2. Performs biocompatibility studies.

1 Galloni M.R., Barberis R.V. *Guidelines for valvular bioprostheses explants studies.* Advances in Biomedical Technology, n. 2, 2008, Zabrze: 109-118.

3. Analyses the explanted devices with the use of the most appropriate procedures: acquisition of accurate macro and micro photographs and X-rays; morphometrical analysis on anatomical samples and biomaterials of both experimental and clinical provenance; histological, histochemical and immunohistochemical protocols, carried out by both routine and innovatory techniques, according to the sample pattern and the study in progress.
4. Provides the results in the form of original certified reports that can be used directly with international Control Commissions.

STUDIES RESULTS

Since the beginning of its activity, Life and Device received about 110 explanted valvular bioprostheses, 10 mechanical valves and 6 mitral annuloplastic rings from different hospitals located in 20 countries. Most of this work was ordered by two major producers.
The analysis of 110 valvular bioprostheses shows that the mean implant follow-up is approx. 3 years. 73% of patients undergoing valve reoperation are female (mean age 54 years) and 27% male (mean age 54 years).
The sewing ring and the stent are covered with fibrous pannus in 76% of cases (mean score 1); this is quite normal, as immobile prosthetic components become physiologically covered by pannus.
Intrinsic and vegetating calcifications are detected in 65% of prosthesis and the mean score is 2 (focal diffuse deposition > of 1 mm).
Tears are present in 52% of explants, mostly along the adherent margin, starting from the free edge near the commissures (score b).
Gram+ colonies are detected in 32% of bioprostheses; the presence of bacteria is also related to thrombus depositions, which are present in 10% of explanted valves.
Cholesterol infiltrations are visible in 14% of cases; this pathology is frequently related to cuspal degeneration.
Inflammatory infiltration is detected in 62% of cases (85% neutrophyles, 68% macrophages and 57% lymphocytes).
After implantation, the histological architecture of the connective stroma, in both porcine and pericardial valves, is modified by mechanical forces during the prosthetic movements; collagen bundles undergo deep modifications, more evident in porcine cusps.
Our activity also deals with preclinical *in vivo* implants, which is an important and necessary step in the development of new devices. We participated in the experimental evaluation of biocompatibility and biofunctionality of a percutaneous bioprosthesis, which involved finding original techniques for the study of non conventional structures.

Great care is always given to the quality of the pictures which, in our opinion, must show with absolute clarity the most significant aspects of the specimens. We do our best to maintain a clear correlation between images obtained with different techniques, such as X-rays, macro, optical microscopy also in polarizing light and different optical contrast methods, scanning electron microscopy.

The Life and Device working team is also involved in the development of new solutions for vascular endoluminal stenting, both in the coronary and peripheral districts.
During the last two years, we examined over 300 experimental coronary stents, both bare and drug-eluting, from pre-clinical trials.
Our team developed specific methodologies in order to be able to study the interactions between biological tissues and the very hard metals of which the devices are made (stainless steel, nickel-titanium and cobalt-chromium alloys).
We improved a plastic embedding technique, utilizing a mixture of n-Butyl and Methyl methacrylate, with Benzoyl peroxide as the catalyst, a medium mostly used in electron microscopy. The desired hardness of the block can be obtained by adjusting the relative proportions of n-butyl and methyl methacrylate; a higher proportion of methyl methacrylate will produce harder blocks that can only be cut with specific microtomes equipped with tungsten carbide steel blades. This technique enables us to obtain very thin and well-preserved slices, that can be stained with any dye.

The whole cycle of our studies ranges from the explant to the compilation of a report which encompasses an accurate macroscopical and histological iconography, as well as anatomical, histological and morphometric analyses, expressed by quantitative and semiquantitative data.
Essential data include lumen area and diameters, neointima, media and adventitia areas, with a thorough description and anatomical scores; neointimal thickness and restenosis rate; injury score; and detailed histological study of neointimal tissue with its inflammation score.

The on-going rapid development of cardiovascular implantable devices and related surgical techniques has created a business that needs an important preclinical step and, subsequently, feedback in the form of explant studies. The mission of Life and Device is to meet the customers' requirements by offering full technical advice, supported by updated scientific knowledge.

The examination of explanted circulatory system prostheses

Jerzy Nożynski
Foundation of Cardiac Surgery Development

1. Biomaterials implanted in the circulatory system.

Prostheses implanted in the circulatory system constitute a rather specific group of materials. They may be made from native or modified human or animal tissue, metallic alloys, polymers or any combination thereof. Every component of a prosthesis, that is a polymer, metallic alloy/metal or tissue, has to possess certain features determining its applicability in a given tissue, such as the heart or a blood vessel, and therefore has to fulfill its physiological function, have an appropriate durability, not cause strain on the surrounding tissue or cause an undesirable reaction in the tissue or the entire system. The above-mentioned features, in particular the absence of toxic effects, immunological response and haemolysis are collectively referred to as biocompliance. The frequently interchangeably used term of biocompatibility has a broader meaning and encompasses not only prosthesis tolerance, but most importantly, the replacement of a part of the body or its function in a safe, reliable, cost-effective, and physiologically acceptable way.

Prostheses and devices implanted in the circulatory system most commonly involve heart valves, intravascular stents and blood vessels. A heart valve must act as a one-way, circular inlet of an appropriate diameter, i.e. one that does not alter the heart's geometry. It should facilitate a correct blood flow, i.e. possess an appropriate opening field. Moreover, it should not react with blood components, i.e. display the tendency to form thrombuses or damage the passing blood cells - cause haemolysis. In addition, it should be durable so as to resist premature damage, whilst its construction should facilitate a technically straightforward and stable insertion into the heart.

Intravascular stent grafts, on the other hand, present a different structural problem. Their role is to restore the patency of a stenosed arterial vessel, usually caused by atherosclerotic lesions. They are produced from metallic alloys and introduced via a catheter, that is minimally invasively. Under radiological monitoring, a stent consisting of a tube-like metal mesh is introduced into the blood vessel via a catheter, then pushed out of the catheter and positioned in the vessel wall. This bioproduct must be flexible and have a shape memory, that is return to its original shape after expansion with no deformation. Like heart valves, stent grafts must not be thrombogenic and have to be non-toxic to the surrounding tissues. They are most commonly implemented in the heart's coronary arteries in order to improve coronary circulation, and in peripheral vessels, such as lower extremity, carotid and intracranial arteries. Aortic stents are used in cases of dissecting aneurysms to provide coverage of this serious lesion in a safe and little invasive way. In recent years, stent grafts have been coated with drug-containing polymers suppressing the artherosclerotic process at the implantation site.

Another example of a circulatory system prosthesis is the Amplatz vascular plug so-called amplatzer, used for minimally-invasive non-surgical closure of heart defects - openings formed as congenital malformation or, most commonly, as a result of infarction of the interventricular septum or the interatrial septum, or the ductus Botalli – a vessel that is essential in prenatal life but whose presence in a growing child or in an adult may lead to circulatory failure. Positioned on a catheter, the amplatzer is intravenously introduced into the heart under radiological monitoring; it then expands – opens like an umbrella on both sides of the defect, adhering tightly. Just like stents, the plugs are made of metallic alloys and must therefore fulfill the same criteria for durability, non-toxicity and stability. The construction of the amplatzer undergoes continuous modifications.

Other circulatory system implants involve short- or long-term electrodes, rings reducing the diameter of atrioventricular orifices and various types of circulatory system/ assistance mechanism combinations.

2. Examination of circulatory system bioprostheses

The clinical examinations performed after the implantation of an artificial heart valve, intravascular stents or a blood vessel segment, assess the post-surgical healing process, the valve's efficiency, and the blood flow in the stent graft or a prosthetic vessel. This information, although essential for the treatment process, does little to reflect all the phenomena taking place at the site of contact between the prosthesis and the patient's tissue, or the changes within the bioprosthesis in general. From a clinical, and even more so, bioengineering point of view, it is important to understand what happens to all the single components of the implanted bioprosthesis and the tissue at the implantation site. This, of course, depends on the particular components of the bioprosthesis. It can be assumed that the majority of metallic alloys used in the production of bioprostheses are chemically and biologically neutral. However, the implantation or the sewing in of the prosthesis is connected with its constant movement, which is transferred via the sutures onto the surrounding tissues. The movement will in a specific way affect the integration of the sutures and the behavior of the

connective tissue, the basic supporting tissue for most organs. What is more, the compression of the surrounding tissue by the valve's ring may also cause local changes. Although the compression of the stent graft in the vascular lumen would undoubtedly improve blood flow, the ensuing formation of expanding tissue would lead to local artherosclerotic lesions. The movable ceramic parts of a heart valve, i.e. the discs, may become the site of adherence of blood cells, thereby leading to the formation of thrombi. The cusps of a biological valve, made of modified and sterile animal or human tissues, would age, and perhaps become colonized with the recipient's cells. Mechanical work, contact with the blood, its proteins and blood cells may cause slight changes on the surface of the ceramic discs. Therefore, it is clear that the longer the functioning time of the implanted prosthesis, the more possibilities there are to observe changes in its structure and its surroundings, all of which constitute crucial feedback for the improved design of new solutions. Hence the need to investigate explanted bioprostheses, acquired during replacement surgery or postmortem. Histological tissue examination facilitates the determination of pathological processes occurring in the surrounding tissue. To this end, micron-thin sections of tissue are assessed under the microscope, following specific staining. An ideal solution would be to obtain cross-sections including the prosthesis margin, however, due to material hardness this is a very challenging task. The commonly used microtome cuts tissues with a knife; however, in order to obtain sections including metal elements, a precision saw has to be applied as it facilitates the sawing off of tissue sections of tens of microns and their subsequent filing to the desired thickness. Accordingly, tissues with soft prosthetic fragments (e.g. fabric-covered or valve cusp tissue) are subjected to histological examinations, whilst hard prosthetic fragments (e. g. the valvular ring and disc) are usually assessed by surface microscopy and mechanical methods, which provide useful bioengineering information.

3. Types of changes occurring in bioprostheses after implantation.

The changes occurring in bioprostheses are similar. Initially, fibrin and platelets are deposited on the biological surface, thereby forming a layer. As in the case of normal lining of heart ventricles, valves and blood vessels (endothelium), blood cells do not adhere to this newly formed layer, which, due to its almost cell-free composition, is referred to as *pseudoendothelium* (Figures 1 and 2).

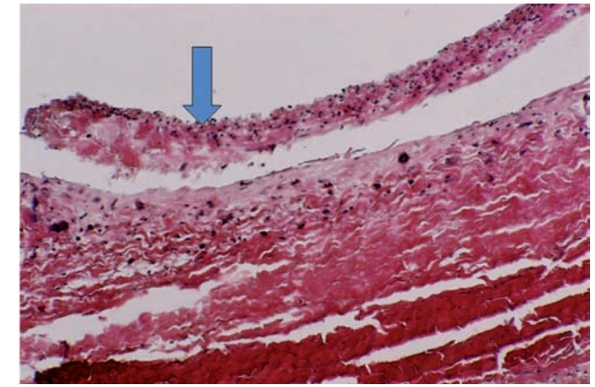


Figure 1. A microscopic photograph of biological valve implantation site. An amorphous pinkish red layer of fibrin with small, dark leukocyte nuclei (*pseudoendothelium*) adheres to the endocardial surface. The layer is unstable and dissects easily (blue arrow). Below is the endocardial surface. H&E Stain. Magnification 100x.

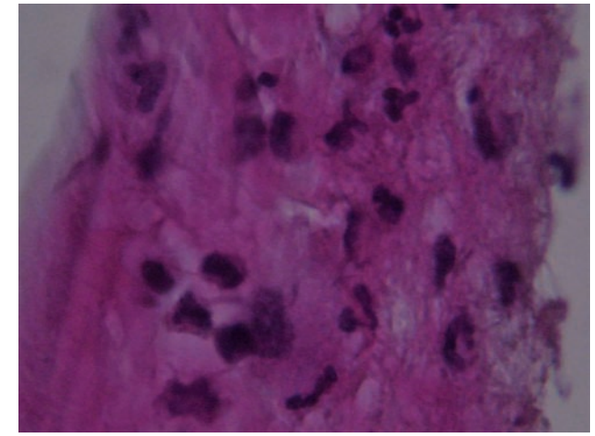


Figure 2. Pseudoendothelium under large magnification. The photograph shows the nuclei of white blood cells and a delicate fibrin net in a pink protein mass. H&E Stain. Magnification 600x.

With the passage of time, the inflammatory response decreases, together with the number of lymphocytes in the tissue surrounding the suture. The number of blood vessels also becomes reduced.

The next stage of healing is the coverage with a layer of endothelial cells (*endothelialisation*) (Figure 3).

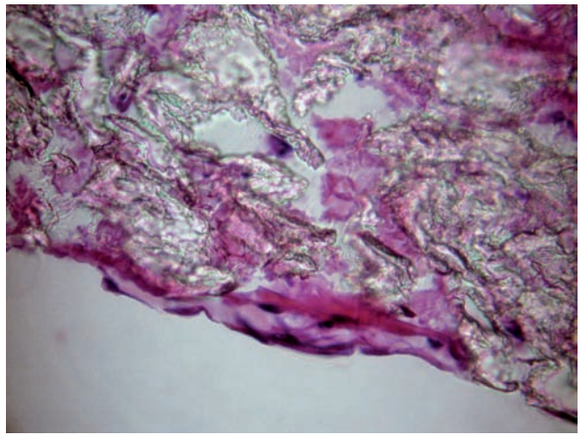


Figure 3. Endothelialisation. A thin layer of pink pseudoendothelium with flattened endothelial cells with dark nuclei adheres to the fibrous surface consisting of birefringent structures saturated with plasma (light pink amorphous masses). H&E stain. Magnification 600x.

This newly formed layer, which is an analogue of the intima of a vessel or the heart, is referred to as the *neointima*. The phenomenon occurs inside stents, on the surface of fabric-covered valve rings and in vascular prostheses. The tissue and the fabric become saturated with plasma.

The healing process taking place parallel at the suturing sites involves the creation of a scar, endocardial connective tissue rich in collagen and, initially, in blood vessels, which stabilises the valve at its implantation site (Figures 4 and 5).

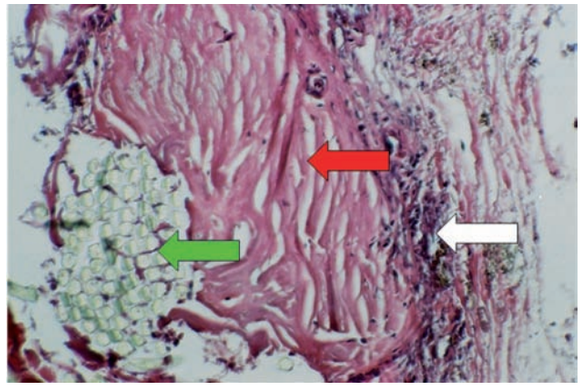


Figure 4. A microscopic photograph representing the endocardial scar formed at the valve implantation site. Green arrow – cross-section through the surgical thread; red – fibrotic endocardium; white – subendocardial inflammatory infiltrations consisting of small, dark staining lymphocytes. H&E Stain. Magnification 80x.

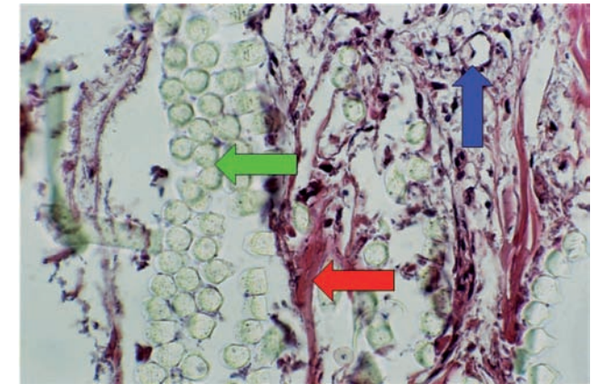


Figure 5. A microscopic photograph of the suture region inside the endocardium. Green arrow – cross-section through the surgical thread; red – connective tissue fibres; blue – a blood vessel. H&E Stain. Magnification 100x.

With the passage of time, the inflammatory response decreases, together with the number of lymphocytes in the tissue surrounding the suture. The number of blood vessels also becomes reduced. The surgical thread is overgrown by connective tissue penetrating its filaments (fibrogenesis) (Figure 6). These cells, the fibroblasts, start to synthesize collagen fibres (collagen biosynthesis), thereby strengthening the newly formed connective tissue scar (Figure 7). In some places phagocytes appear, which, by eroding the connective tissue elements, model the connective tissue around the suture. Scar modelling is a long-term process and, just like processes in other tissues aiming at the adjustment of the structure and composition to an organ's current function, it is referred to as remodelling.

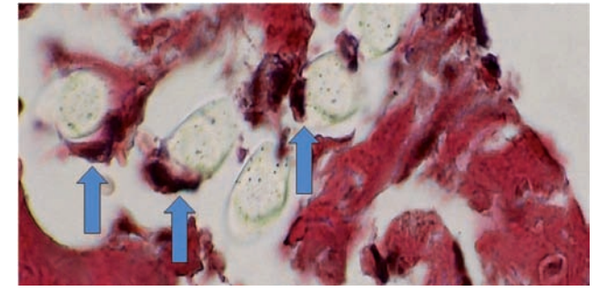


Figure 6. A microscopic photograph showing surgical sutures in the endocardial scar – fibrogenesis. Blue arrows point to fibroblasts between the filaments of the surgical thread (birefringent, partially transparent ovals). Connective tissue cells combine with the pink stained connective tissue fibres. H&E Stain. Magnification 600x.

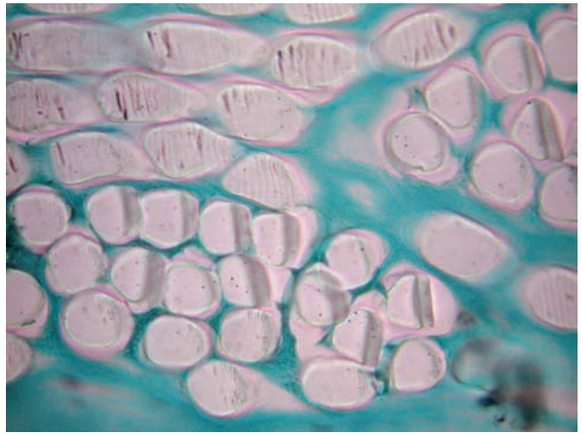


Figure 7. A microscopic photograph of surgical sutures in the endocardial scar – fibrogenesis in specific staining. The greenish collagen masses penetrate the filaments of the thread. Masson's Trichrome Stain. Magnification 600x.

As the collagen forming the collagenic scar penetrates the fibres of the myocardium, the scar is not limited to the endocardium only, but partially encompasses also the myocardium (Figure 8).

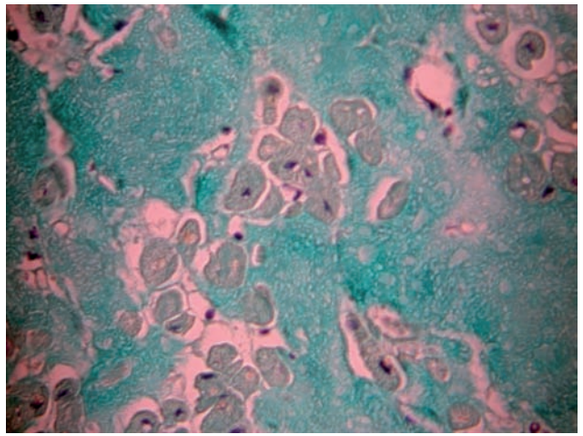


Figure 8. A microscopic photograph of surgical sutures in a scar – fibrogenesis in specific staining. The greenish collagen strands penetrate between the fibres of the myocardium. Masson's Trichrome Stain. Magnification 200x.

The healing process of the myocardium following a cardiosurgical procedure has not been extensively described in literature. At the implantation site, this process involves the removal of small necrotic lesions formed during the surgery. Therefore, it is also connected with the presence of infiltrations consisting of

inflammatory cells, lymphocytes and phagocytes (macrophages - histiocytes), which may sometimes fuse, thereby creating polynuclear cells (histiocytes around a foreign body) (Figures 9 and 10). The presence of surgical threads can also cause an inflammatory reaction as the phagocytes attempt to consume the threads, which, naturally, should not happen.

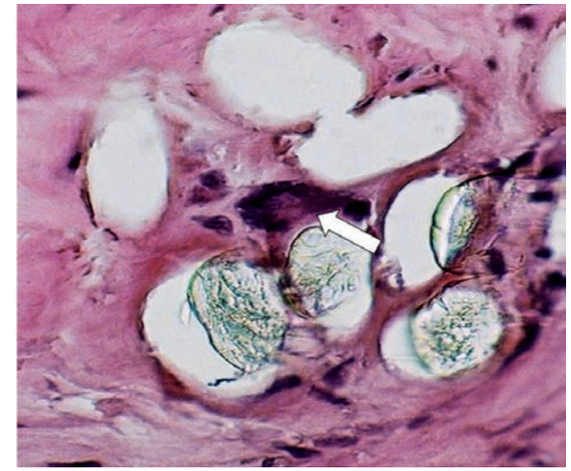


Figure 9. A microscopic photograph of surgical sutures in the endocardial scar. The arrow points to a polynuclear histiocyte with multiple blue nuclei typically arranged in a horseshoe pattern. H&E Stain. Magnification 600x.

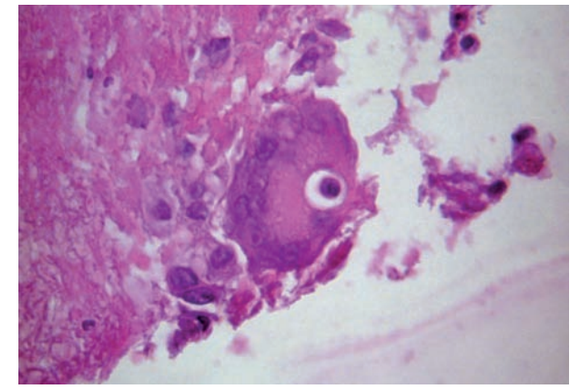


Figure 10. A microscopic photograph showing a giant cell found in the endocardial scar (centre of the photograph). The nuclei are arranged in a horseshoe pattern or, mores precisely, a C-shape. The cell contains a vacuole with digested residues inside. The cytoplasm is slightly foamy and is surrounded by histiocytes. H&E Stain. Magnification 800x.

The creation of blood vessels in the endocardium, referred to as vascularisation, is often disregarded. However, it is undoubtedly related to the nutritional

demand of collagen producing cells, i.e. fibroblasts in the forming scar (Figure 11).

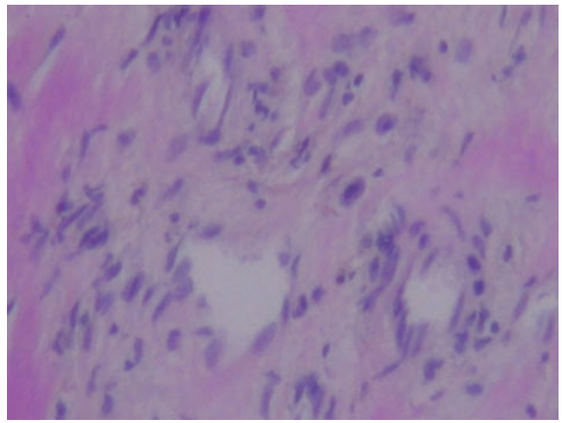


Figure 11. A microscopic photograph representing a fresh endocardial scar. The multiple oval, purple fibroblast nuclei are well visible on the lighter background of the connective tissue. On the left, pink fibers of the myocardium. There are cross-sections of vessels visible in the connective tissue. H&E Stain. Magnification 250x.

Fibrogenesis, i.e. an increased collagen production, is particularly visible in the endocardium of the common atrium after the implantation of an atrioventricular valve. The endocardial stratum appears to be an acellular substance, which after specific staining, reveals multiple, green-stained collagen fibres of varying thicknesses (Figure 12).

Figure 12. A microscopic photograph showing endocardial fibrosis of the left atrium after mitral valve implantation. Visible greenish collagen fibres. Masson's Trichrome Stain. Magnification 100x.

There are foci of calcification between collagen fibres in the endocardium. In our observation, they are present in over 50% of cases (Figure 13).

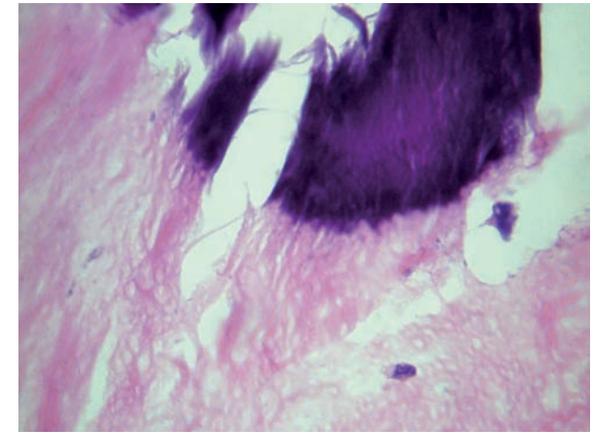
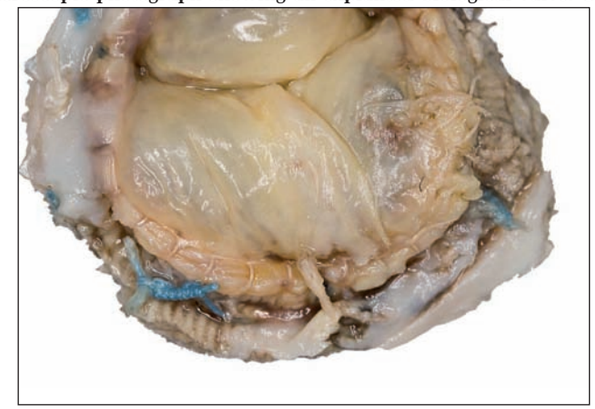


Figure 13. A microscopic photograph representing endocardial fibrosis of the left atrium after mitral valve implantation. The collagen fibres are pale pink, whilst calcium deposits are intensive purple. H&E Stain. Magnification 200x.

Occasionally, the formation of endocardial connective tissue goes beyond the physiological limits and becomes a progressive, collar-like hyperplastic process obscuring the valve opening area. This phenomenon, known as the pannus, is a rare complication following valve implantation, most frequently occurring in experimental prosthetic valve implantation in animals (Figures 14, 15 and 16).

Figure 14. A microscopic photograph showing an explanted biological valve. On the outside,



there is the fabric which covers the valvular ring, inside which there are the blue threads of the suture. On the inside, the convex valvular cusps made of modified tissue are visible (photo by M. Jakubowski).

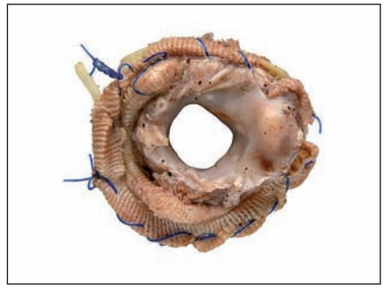


Figure 15. A microscopic photograph of an explanted biological valve. On the outside, there is the fabric covering the valvular ring, inside which there are the blue threads of the suture. On the inside, a white-grey funnel-shaped collar which narrows the valvular lumen, is visible (pannus). The cusps of the valve are translocated downwards and ingrown with the connective tissue (not visible in the photograph) (photo by M.Jakubowski)..

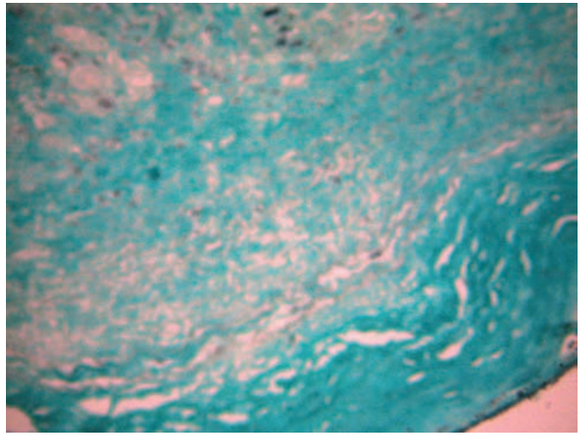


Figure 16. A microscopic photograph showing the pannus. Practically, there are only thick strands of green-stained collagen and few dark cellular nuclei. Masson's Trichrome Stain. Magnification 80x.

The collagen scar undergoes a transformation during the ageing process. The collagen fibres group to form thick strands; the fibrous system subsequently undergoes homogenisation, resulting in the so-called hyaline degeneration of the connective tissue. As a result, a strong, thick and slightly fibrous structure is created (Figure 17).

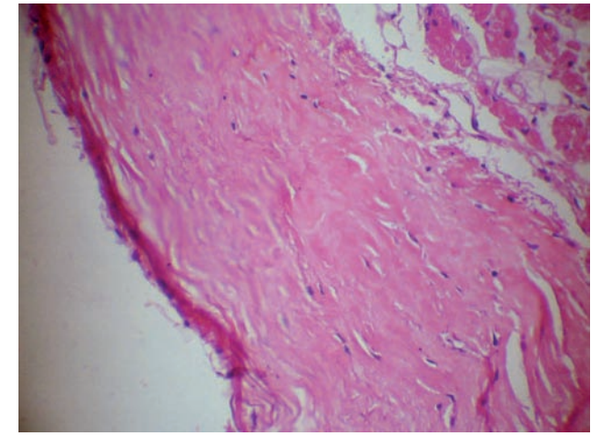


Figure 17. A microscopic photograph of the endocardium with features of hyaline degeneration - hyalinization. A thick pink layer with a slightly marked fibrous pattern, clefts and few cells. H&E Stain. Magnification 80x.

Granulocytes are present only in cases complicated by the presence of pathogenic bacteria. Bacterial endocarditis may spread from the cusp of the bioprosthesis to the surrounding implantation site, leading to the weakening of the suturing sites and the formation of paravalvular leak. The surface of biological valve cusps, changed as a result of degenerative or inflammatory processes, frequently becomes the adherence site for platelets and blood cells, which form there thrombuses that immobilise the cusps or constitute a potential source of embolisms (Figure 18).

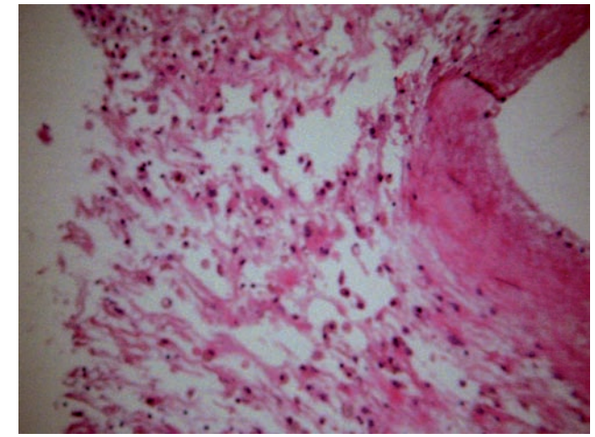


Figure 18. A microscopic photograph of the endocardium with bacterial inflammation. An extensive inflammatory infiltration in the subendocardial connective tissue. The dark nuclei of lymphocytes and granulocytes are clearly visible. H&E Stain. Magnification 80x.

The question of changes occurring in the animal or human tissue of biological valve cusps is very challenging and has frequently been described. A sterilized or modified tissue contains either a very small number of living cells of the connective tissue - the fibroblasts, or has no living cells at all, therefore it is not possible to reconstruct its skeleton – the connective tissue fibres. When saturated with plasma, the cusp of a biological valve gradually loses the collagen scaffold as a result of fibre decomposition. Its structure becomes homogenized, leading to long-term progressive degeneration, calcification or other changes connected with mechanical material fatigue, such as: cusp stretch, dissection, shortening or rupture, and eventually valve insufficiency. The colonization of the valvular cusp by pathogenic bacteria, leading to bacterial endocarditis, is a very serious complication.

Advances towards understanding complications of cardiac valve prostheses

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It is known that approximately 60% of substitute valve recipients develop prosthesis-related complications within 10 years postoperatively. These complications differ significantly among types of artificial valves. The most often complication finds in mechanical valves for example is obstruction of it by thrombus. Another serious complication is infective endocarditis or paravalvular leak due to inadequate healing. Special attention in this review will be pay to the valve obstruction due to overgrowth of fibrous tissue during the healing process. The mechanisms of such overgrowth are still open to question. Accordingly, we hypothesized that at least two conditions may influence on the overgrowth of fibrous tissue in the artificial valves. First, mechanical stress after implantation of the artificial valve may stimulate collagen synthesis by fibroblast and/or myofibroblasts; second, the induction of chronic immune response (reactivation of inflammatory conditions) may occur after surgery influencing on matrix valve remodeling.

It has been described that interstitial cells form all valve types express muscle structural genes including the cardiac isoforms of troponins, beta-myosin heavy chain and myosin light chain. In about 57% of these cells presented alpha-smooth muscle cells staining in the immunohistochemistry study indicating myofibroblast etiology. Interstitial cells are responsible for matrix secretion and remodeling. In addition, the valve interstitial cells play a significant role in valve repair including migration, proliferation, apoptosis, necrosis and calcifications.

Several clinical studies reported that interleukin-6, proinflammatory cytokine increased markedly after surgery, peaking 6 hours after surgery. In a second phase, CRP levels were increased. In addition, approximately 20-40% of patients surgically treated developed within 2 - 3 days atrial fibrillation being proinflammatory condition. It is worth emphasised that valvular heart disease is an independent predictors of elevated CRP level being marker of systemic inflammation.

The study of valve pathology showed that degenerative and inflammatory conditions are most often associated with accumulation of valvular interstitial cells and matrix, inflammation, and calcification. Inflammatory cells most

often found were macrophages and lymphocytes. Prominent inflammatory infiltrates are seen in chronic rheumatic valve disease and in areas of prominent calcifications. Our study of explanted artificial valves suggests that besides macrophages, lymphocytes, and valvular interstitial cells, the mast cells may play a significant role in matrix synthesis and remodeling, as well.

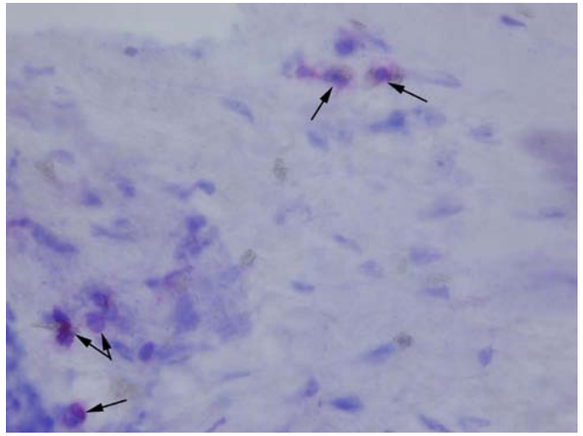

Fig. 1. The activated lymphocytes CD3(+) (arrows) observed in cryosection of explanted valve.

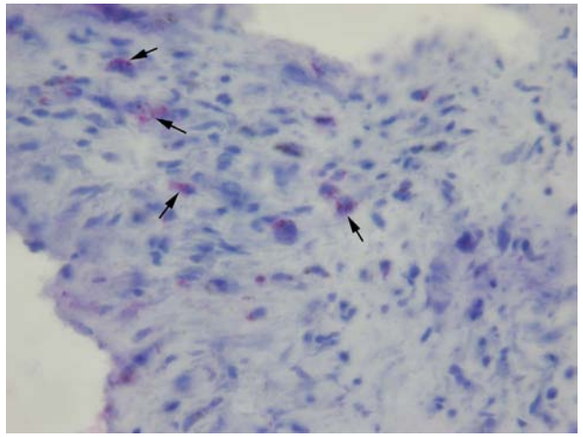

Fig. 2. The CD68 positive cells (arrows) present in the cryosection of explanted valve.

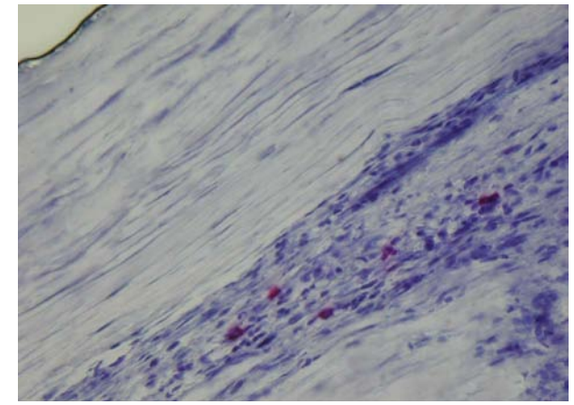

Fig. 3. A few mast cells (tryptase positive)(red color) in high-cellular area of valve section.

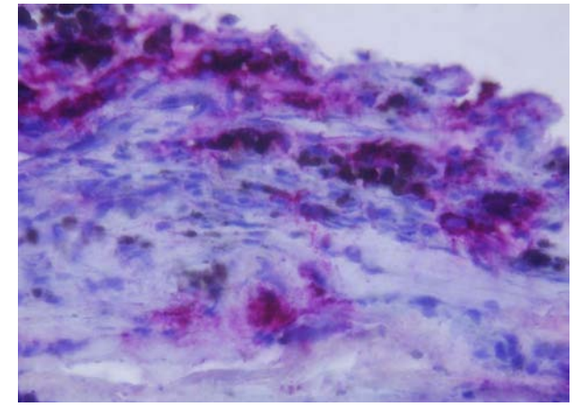

Fig. 4. Numerous mast cells (red color) in another cryosection of explanted valve.

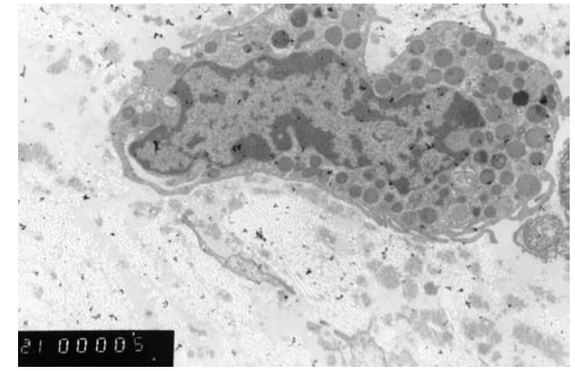

Fig. 5. Electron microscopy study revealed mast cell surrounded by dense collagen fibres.

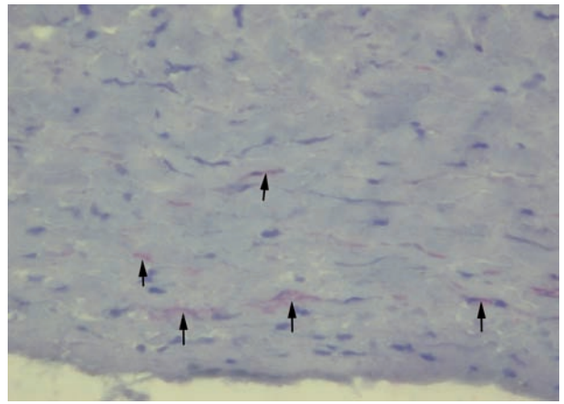

Fig. 6. VEGF-positive staining (arrows) for small capillary vessels of tissue from the explanted valve.

Concluding, it is highly likely that mast cells play a significant role in matrix remodelling presented as overgrowth of fibrous tissue in the artificial valves.

Nanostructural materials for implants and cardiovascular biomedical devices

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Abstract

Modification of implants on the external surface for improving biocompatibility, especially in contact with blood, was the main objective of the work. Studies of new tissue contacting materials were focused on blood contact aspects. Materials for external and internal blood circulation support devices are planned to be elaborated. Surfaces of the implant devices were covered with non-cytotoxic and anti-thrombogenic coatings biomimeticly behaved to its surrounding e.g. vessel surface, thus influencing the endothelial cells to cover. Depending on the topography of the surface and the chemical nature of the cells a nonthrombogenic bio-surface was formed, basing on the covered polymer by cells. Carbon, titanium and biopolymer based materials were examined. Therefore, it was used plasma as pre-treatment in vacuum by coating. State-of-the-art devices are polymer tubes used for forced external and internal blood circulation. They are normally internally modified with heparin bond, preserving against the clot formation in blood contact. By lowering the thrombogenicity, the increasing risk of bleeding is the main disadvantage of these systems. Thus, a main concept of this work was to improve the surface-blood cell interaction.

1. Introduction

Synthetic materials which are widely applied for the vascular reconstruction are strength and seem to be useful for medical applications, however, a high stiffness makes the serious problem. It causes the very low blood flow, especially in the low diameter vessels, and moreover, leads to a long term blood- material interaction, platelets adhesion and their activation. When the clot formation cascade begins, it could finally close the vessel light. The other aspect coming from the artificial material interaction with the natural tissue is disconnection

between the tissue and an introduced material. The above mentioned disadvantages are caused by a lack of the negatively charged endothelium layer eliminating clotting processes by formation of non thrombogenic, semi-leaking membranes, which are responsible for secreting anti- thrombogenic compounds which take part in the vessel reconstruction processes. The endothelium cells are responsible for interaction with blood cells as well as they are necessary to keep the permeability of the vessels.

Thus, the tissue-engineered vascular grafts are optimized by researchers around the world to make them similar to the natural vessels. The angiogenesis (natural vascular production) is the main natural pattern among the natural processes which is taken under the consideration. The most important steps are as follows:

- angiogenetic process stimulation- VEGF,
- proteolitic enzym stimulation,
- cell adhesion and migration,
- cell maturation,

The main idea is presented in Fig. 1.

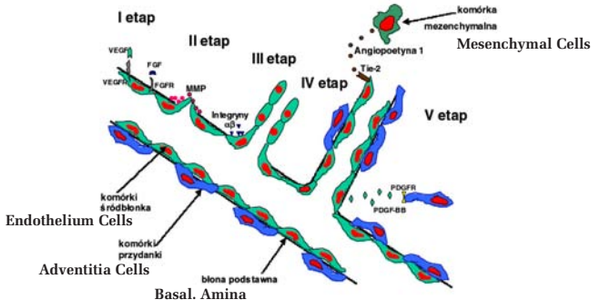

Fig. 1. The main mecha

The presented work is focused on elaboration of appropriate materials for scaffolds by endothelialisation and fictionalization using the tissue deposition. The key issue in this content based on the basic assumptions of the tissue bioengineering. The tissue formation triangle is considered and it is shown in Fig. 2.

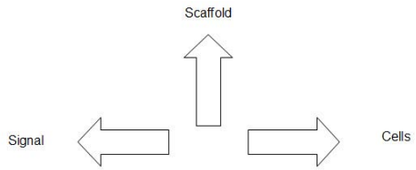

Fig. 2. Main assumptions of the tissue formation

The first step to optimize the process of the artificial vascular prostheses design is formation and elaboration of scaffolds and understanding the blood- material interaction. The initial reaction which takes place at a contact of blood with foreign material is the protein adsorption from the serum. The most probable proteins are: albumins, fibronectine, fibrinogen and immunoglobulines G.

The goal of this work was fabrication and functionalization of new tissue contacting materials by formation of bioactive surfaces.

2. Film fabrication and surface functionalization

2.1. Thin coatings fabrication

Films were fabricated by means of the surface modification dedicated to the blood-material interaction purpose. Materials were elaborated by magnetron sputtering in direct current (DC), unbalanced mode. Titanium (medical grade titanium for titanium and titanium nitride coatings) and carbon targets (for Diamond Like Carbon-DLC) were used to deposit 10-20 nm thick films on polyurethane substrates at room temperature in an argon atmosphere (diamond-like carbon (DLC) and titanium (Ti)) or in a nitrogen-argon atmosphere (titanium nitride (TiN)). To ensure a homogenous film thickness over the entire coated surfaces, substrates were rotated during deposition at a speed of 5.4 cm s-1 through the plasma plume. A detailed description of the deposition arrangement is given elsewhere [2,3]. There were applied industrially up-scaled coating processes at JOANNEUM RESEARCH Forschungs-GmbH, MATERIALS UNIT, which allow coating of polymers at the room temperature and 3-axis substrate/planetary rotation. One of the main success was to elaborate ceramic layers exhibiting an elastic behavior. One could ask; how it was possible? The answer is associated with the appropriate structure and the proper mechanism of the thin film nucleation from the gas phase (Fig. 3) [4-18]. The cross section of the titanium nitride coating is presented in Fig.4. The possible explanation is presented in Fig. 5.

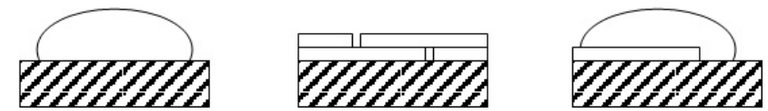

Fig. 3. Cross-section views of the three primary modes of thin film growth including (a) Volmer-Weber- island formation, (b) Frank-van der Merwe- layer-by-layer, and (c) Stranski-Krastanov-layer-plus-island

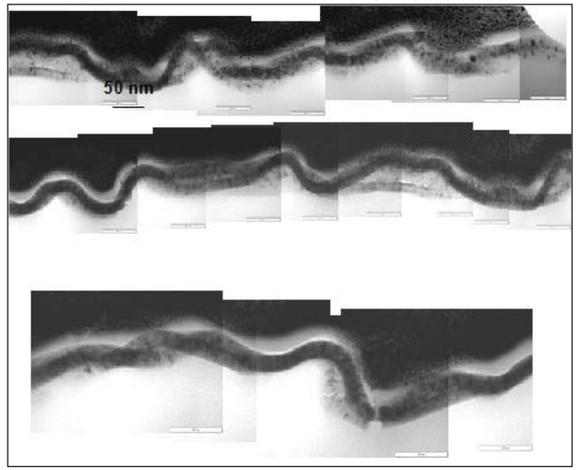

Fig. 4. Transmission electron microscopy (TEM) microstructure of cross section of the titanium nitride coating

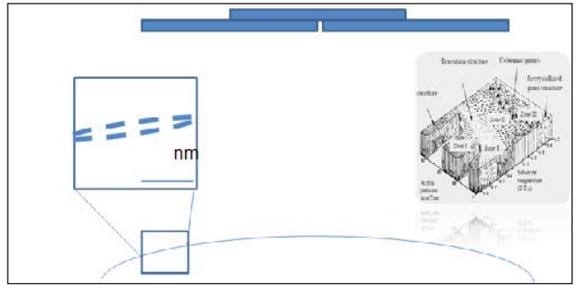

Fig. 5. Possible explanation of the elastic behavior of the thin coatings

The theoretical explanation of the observed phenomena is presented in Fig. 3. It is connected with the mechanism of the thin film growth [5]. The layer-by-layer model gives the structure and properties which finally allows the material fabrication. Between the each individual sub-layers the nano-cracks are possible to be formed. Nano-cracks in fact are visible as a material straggling. It does not eliminate it for the biomedical use. Each individual sub-layer strongly adhere to the substrate, moreover, the nanodiscontinuity of the coating are covered by the upper sub-layer. The deposited layer did not exhibit diffusion character in respect to the substrate. It is impossible because of the physical process and it could not take place due to the polymer substrate. Only pseudo-diffusive character could be considered which was confirmed by a high resolution transmission electron microscopy technique. The anchoring mechanism was observed (Fig. 6).

The other aspect of the proper and unique behavior of the ceramic coating comes from formation of a close to amorphous structure.
Thin coatings, which exhibit good adhesion, biocompatibility and a lack of substrate degradation, was the key issue of the first part of the work. The following paragraph (2.1.1) will consider these three aspects of the blood-contacting material design and its elaboration.

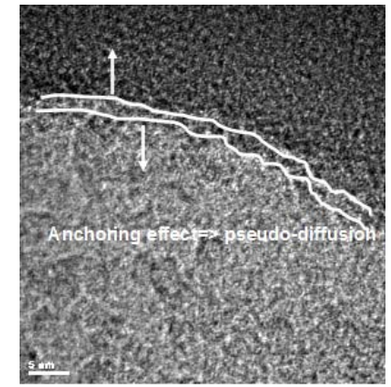

Fig. 6. The high resolution electron microscopy micrograph

2.1.1. Scratch and indentation tests

Adhesion of coatings to polymer substrates was measured by the micro scratch technique using a Rockwell C spherical diamond stylus (cone apex angle 120°, tip radius 200μm). Tests were done under increasing load from 0 to 10N, within the distance of 5mm. For ceramic coatings neither cracks nor delaminations were observed. It should be pointed out that failures of coating-substrate systems have not been appeared although the penetration depth corresponding to the maximal load was 100 μm that is many times higher than coating thickness. This implies a good adhesion of those coatings. For the Ti thickest coating, small areas were removed under load 3N (Fig. 7). Evolution of the friction coefficient against diamond indenter during the scratch test (load range 0-5N) was shown in Fig. 8. The lowest friction force was measured for TiN coating (μ=0.25- 0.28). Slightly higher value of the friction coefficient about 0.35 was observed for Ti(C,N) coating. In comparison with ceramic coatings, the Ti metallic coating has higher friction coefficient (μ=0.5). That is caused by the contact of indenter with a polymer substrate at areas where coating was removed. For all examined systems, the penetration depth measured during the scratch test was similar and determined by deformability and ploughing of soft polymer substrate. Micrographs of the scratch trucks of all coatings were shown in Fig. 7.

Fig. 7. Light microscopy (LM) micrograph of the scratch trucks for coatings: a) Ti, b) TiN, c) Ti(C,N)

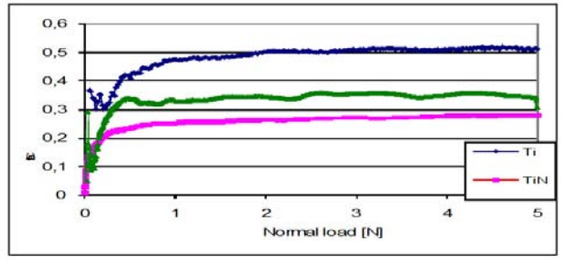

Fig. 8. Friction coefficient measured during scratch test for: a) Ti, b) TiN, c) Ti(C,N)

A spherical indentation technique with 20μm indenter radius was used for determination of the strength and fracture of tested samples. The obtained penetration depth-load curves for all systems within 0-200mN load range were compared in Fig.9. The maximum penetration depths were again many times higher than coating thickness. The highest penetration depths were measured for Ti coating, that shows the lowest stiffness of this system caused probably mainly by the lower Young's modulus of titanium coating and cracks exists in Ti coating. No pop-ins on penetration curves for TiN and Ti(C,N) were observed. These jumps of penetration depth normally appear at the moment of coating fracture. Deformations were mainly elastic with residual depth lower than 15% of maximum depth. Fig. 10 presents corresponding stress-strain curves from spherical indentations. Coating influence on deformation is clearly visible to 0.02 strain, that corresponds to 500nm penetration depth and 5mN load. The higher stress for ceramics coatings is caused by their higher stiffness (Fig. 10). For Ti coating initial stress is lower but their highest thickness caused stronger influence on deformation of polymer-coating system. For ceramics coatings drop of stress is more rapid than for titanium layer. For strain higher than 0.02 substrate controls deformation of all tested systems.

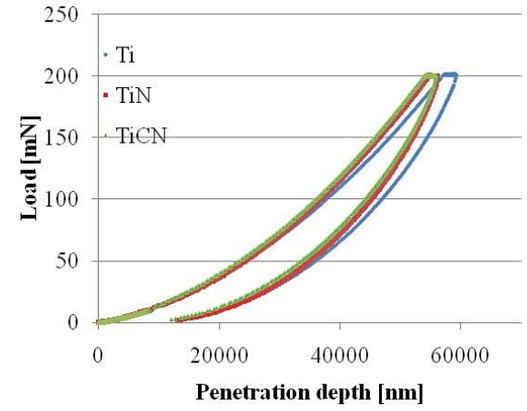

Fig.9. Indentaion curves for Ti, TiN, Ti(C,N) for spherical indentations with 20μm indentor radius under 200mN maximum load

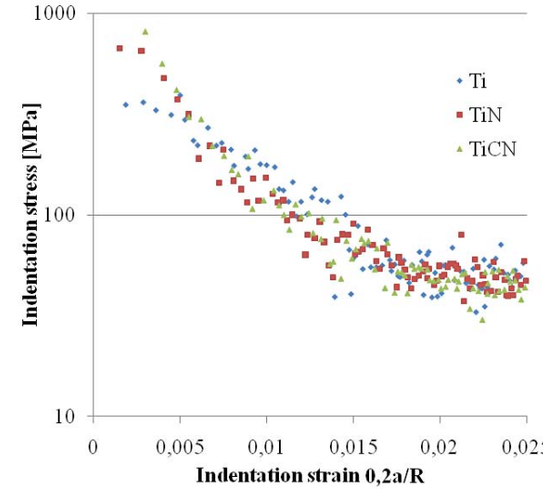

Fig.10. Indentation stress-strain curves for spherical indentations

2.1.2. Thermal effect

Temperature and atmosphere in the reactive chamber seem to be the basic parameters which determine the proper structure. The temperature effect was analyzed as the heat flow in the function of the applied temperature (Fig. 11). The performed investigations realized small glass transformation on the level 110 deg. All visible effects came probably from the substrate. Materials which were applied for the surface modification exhibited various reactions:

- Ti, Ti(C,N), TiO, DLC+Si caused additional endothermal effect in the lower temperature area
- TiN, DLC, DLC+Ti, influenced on the additional endothermal effect but the change of the temperature of the glass transformation is visible as well.

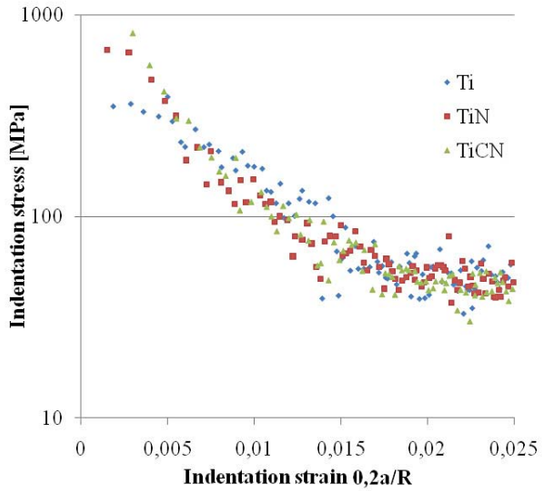

Fig.11. Heat flow in the function of the applied temperature

2.2. Cell- material interaction

This chapter presents the biocompatible tests performed on the investigated materials in static and dynamic conditions. There were considered two important aspects in the biomaterial design i.e. stoichiometry and phase composition modification with carbon introduction.

For the preliminary study of cell–material interactions, the mouse fibroblast cells line L929 from ATCC collections was used. Cells were cultured in "Medium 199" supplemented with 10% FCS (Fetal Calf Serum) and with antibiotics streptomycin and penicillin (Sigma) at 37 ^{O}C with 5% CO_2 in a humidified incubator (Haereus). The media were changed every 2 days. Cells were cultured in 25 cm_2 culture flasks. Media were changed every 2 days. When L929 cells in a culture were grown to 75% confluence, the cells were ready to use for the cytotoxicity tests.

Before the tests, samples were subjected to a sterilization process using ethylene oxide. Afterwards, they were ventilated for 2 weeks to remove rests of ethylene oxide. Before seeding, the cells were harvested from the culture dishes using 0,05% Trypsin/EDTA solutions and re-suspended in a culture medium (Medium 199 supplemented with 10% FCS, 1% L- glutamine) to the concentration

of 5 x 10 ^{6} cells/ml. The tested materials were cut into squares and placed in 24 wall culture dishes. To ensure a complete contact between the samples and wall, the samples were pressed with plastic rings. Then the pellet of re-suspended cells were placed on the material surface with a density of the cells around 10 000 cells/well. After 30 min of incubations, the culture media were completed to a total volume of about 3ml/well. An empty well with fibroblast cells was used for control. The materials with cells and control were cultured at 37°C with 5% CO_2 in a complete medium (Medium 199 supplemented with 10% FCS, 1% L- glutamine) for totally 24h.

To observe changes in the cell viability and morphology after cell material interactions (attachment), we applied fluorescent microscopy. Using the Live/Dead dye Fluorescence Diacetate (FDA) and Propidium Iodide (PI) the viability of cells was analysed. Viable cells stained green with FDA and non-viable cells stained red with PI.

The stoichiometric titanium nitride is known as biocompatible material. A lack of stoichiometry could result in a negative influence on the surrounding tissue. To explore this problem, both groups, the biocompatible and not biocompatible titanium nitrides were elaborated.

Fig. 12. presents results of biocompatibility tests of the example material. For this purpose titanium nitride (TiN) was considered. The experiments revealed that the cells exhibit high biocompatibility on the surface of the fully stoichiometric TiN (Fig.12). It is visible growth in cells and spread across the surface, no influence on the cell death was detected.

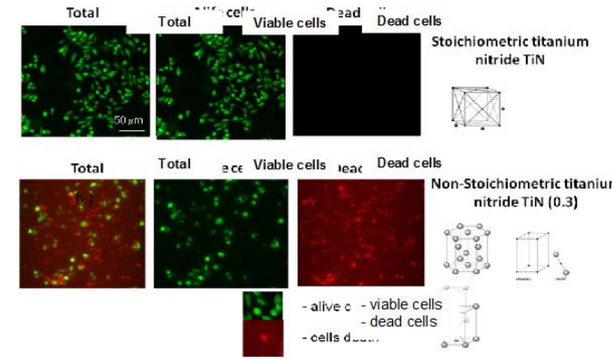

Fig.12. Titanium nitride (TiN) structure influence on the cell growth and proliferation a.) stoichiometric TiN b.) TiN 0.3- turbostratic structure

Titanium nitride could be modified with carbon introduction into the surface. The technique is complicated, because of the two different reactive gases mixture (acetylene for the carbide and nitrogen for the nitride) with neutral

gas (Ar) in addition. There is well known that the carbon should influence on the positive cell-material interaction. The aim of this part of the work was to follow the idea and the task associated with the titanium nitride and to compare the cell-substrate reactions depending on the carbon introduction under hydrodynamic conditions (radial detachment test). The radial shear flow test was used to determine the efficiency and kinetics of cell detachment from plain as well as modified surfaces as a function of applied shear stress. The investigated materials were considered in the individual conditions described above. Depending on the material different exposition time was necessary. The results of the cell rate detachment under the applied shear stress are presented in Fig 13.

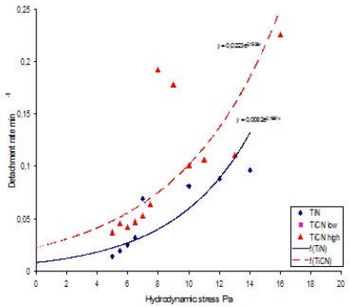

Fig. 13. Detachment rate as a function of hydrodynamic stress correlated to the distance from the center of the test system

TiN exhibits low cell-material interaction. Detachment rate for the titanium carbonitride with the low carbon content was over estimation, thus it was not presented on the diagram. Values of the threshold stress confirm the observations of the cell distribution. The lowest value 3Pa typical for TiN, the highest for TiCxNy with the high carbon (high C) content 7Pa. Function extrapolation for the 0 Pa hydrodynamic stress revealed spontaneous cell detachment rate 0.02 [1/min^{-1}] for Ti(C,N) and 0.008 [1/min^{-1}] for TiN. The performed cell detachment test gives the overview about the cell behavior and shear stress which could appear between the cell and the biomaterial. But now the question appears, how the cell-material interaction looks like for the full group of the coatings mentioned in the experiment description. The results are shown in Fig. 14.

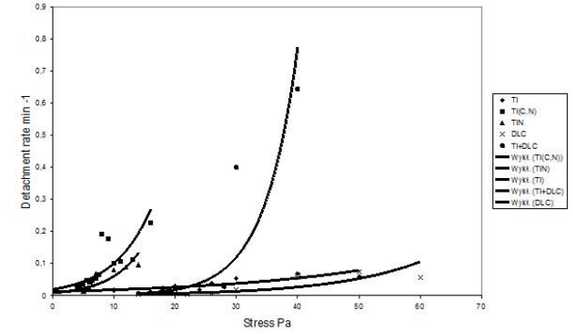

Fig. 14. Detachment rate as a function of hydrodynamic stress correlated to the distance from the center of the test system- experiment for the wide range of layered materials

It is clearly visible that there are differences between the nitrides, carbides and metallic coatings. Titanium nitride (TiN) and titanium carbonitride (Ti(C,N)) exhibit lower cell- material interaction comparing to diamond like carbon (DLC), titanium doped diamond like carbon (Ti+DLC) and titanium (Ti). The function extrapolation to the 0 Pa shear stress allows to estimate the spontaneous detachment rate, without the shear stress. Results are presented in Table 1.

Table 1. Spotnaneous detachment rate

Material	DLC	Ti(C,N)	Ti	Ti+DLC	TiN
Spotnaneous detachment rate [min^{-1}]	0.011	0.022	0.0081	0.0004	0.0082

Biomaterial design requires the biophysical tests as well as biocompatible tests which would illustrate the biological response of the biomaterial to the biological cells. In this case the tests are performed in static conditions. The second aspect of the cell- material interaction revealed the blood- material interaction (BMI) tested under simulated vascular flow conditions. The Impact-R test is a novel device for testing platelet function under close to physiological conditions. The device tests platelet adhesion and aggregation in an anti-coagulated whole blood (citrate buffer tubes) under arterial flow conditions (1800 s $^{-1}$; for 2 min).
Furthermore, it provides a quick method for monitoring the response to various antiplatelet drugs. The detailed information is presented in the literature [19-23]. In the Impact-R test PTFE cone (2.45° point angle) rotates 5 min in a well with a polystyrene (PS) base plate with 130 μl dispensed venous blood to achieve constant shear rate (1800 s^{-1}). For testing biomaterial thin films, PS plate was replaced by coated (and for reference uncoated) PU discs.

For Impact-R the full spectrum of the layer materials were tested: diamond like carbon (DLC), metallic titanium (Ti), stoichiometric titanium nitride (TiN), titanium carbonitride (Ti(C,N)), titanium oxide (TiOx), silicon doped diamond like carbon (Si+DLC), titanium doped diamond like carbon (Ti+DLC). Blood was taken from male healthy donor, 50 years old. Baseline control of platelet function was analyzed by static storage and full activation by ADP (adenosine diphosphate). Platelet ADP receptor plays a pivotal role in serum-induced platelet activation/aggregation. The following aspects of BMI were analyzed:

- the aggregates: platelet- monocytes, platelet- neutrofiles and platelet-platelet
- the change of the integrin receptor conformation

On the surface of the platelets there are the appropriate receptors, which take participation in the adhesion and aggregation. There are mainly selectins, responsible for the initial stages of the adhesion process. Selectin P which is typical for the blood platelet is a glycoprotein, it is accumulated in the platelet granules and transported to the membrane after platelet activation [24].

The platelet-platelet aggregates were consider as a big aggregates (over two platelets) and small ones (two). Results of the amount of the aggregates as a function of the applied material are presented in Fig. 15.

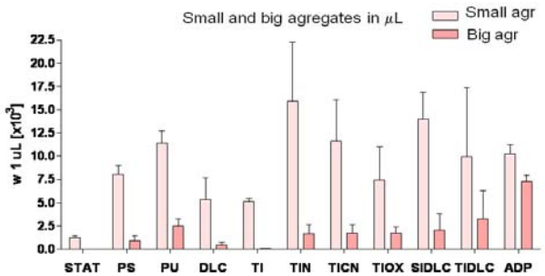

Fig. 15. Percentage of the remaining cells as a function of distance

The results should be read in the reversed way. The presented values consider the blood taken from above the sample. Finally, the smallest amount of aggregates is visible for TiN, Ti(C,N) and Si+DLC. The results were confirmed by the calculation of the amount of platelet aggregates as a function of amount of platelets (Fig. 16).

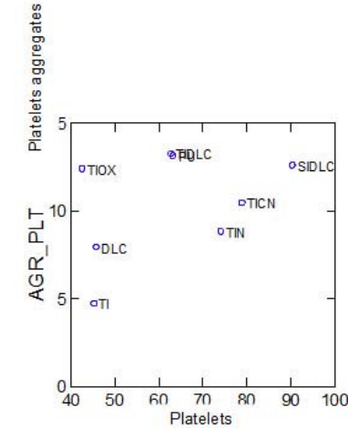

Fig. 16. Amount of platelet aggregates as a function of amount of platelets

The differences between the coatings were found according to the ability of the platelet adhesion. For Ti, TiOx and DLC the highest platelets use was found. For Ti and DLC also platelets aggregates were adhering, thus their decreased number and small activation parameters PAC1 i SelP. TiN, Ti(C,N) i Si DLC caused the smallest platelets degradation. Concerning amount of platelet agregate number and the platelet activation, the best biocompatibility was found for Ti(C,N) and Si+DLC. The coating Si+DLC gives less platelet-granulocytes aggregates.

2.3. Surface functionalization

2.3.1. Migration channels

The second attempt dealing with the cell- material interaction was focused on the surface functionalization. It should lead to the appropriate cell behavior and gives the designers the opportunity to have an easy control on the cell activation or deactivation. Our aim was to produce the endothelium layer on the surface dedicated to a blood-material interaction. Extracellular matrix (ECM)-like biomimetic surface modification of cardiovascular implants is a promising method for improving hemocompatibility. The preliminary attempt considered the migration channels. The idea was to have an influence on the cell adhesion and proliferation. Thin coatings were deposited using hybrid technique, based on physical processes. Furthermore, the channels on the half thickness of the deposited coating were elaborated. The channels were prepared using laser ablation technique (Fig. 17).

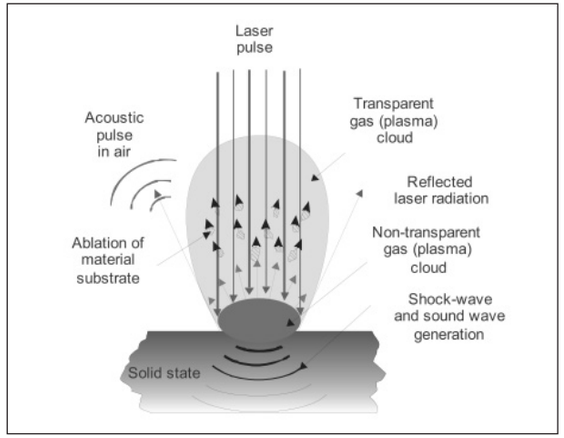

Fig. 17. Pulsed laser material interaction processes

One should consider several physical phenomena which take place during the process performance. They all have an influence on the quality of the final product. The process is widely described in literature [25].
Migration channels were prepared and Fig. 18 presents the topography of the surface after their fuctionalization.

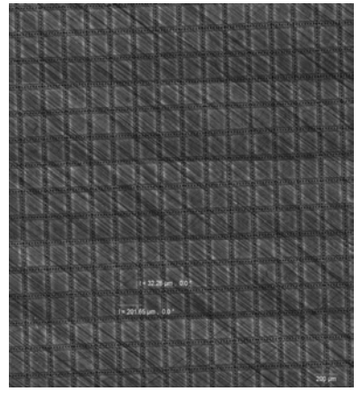

Fig. 18. Functional surface, prepared by the laser ablation techniques for the cellular migration purposes

The surface topography was analyzed using confocal microscopy working in a topography mode. The channels were not free from disadvantages. It was revealed that the pulsed laser beam caused the additional wells and gates formation in the channels (see Fig 19). Line scan exhibited the hills on their border. It was the result of the re-sputtering effect during the channels

formation. More information, describing the mentioned processes, are presented in literature [2,3,25].

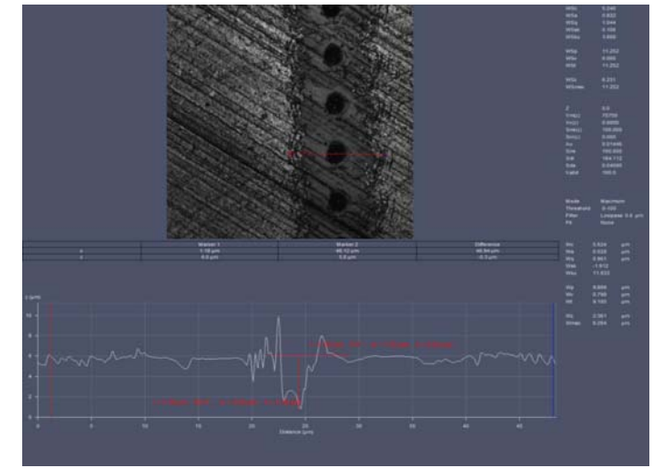

Fig. 19. Topography investigation of the migration channels

New opened questions appeared. Whether the cells will migrate? Will they consider the chemistry or the surface topography change? Will they see the wells?
Experiments of the cell migration were set to find the answer for the remained questions. The results are presented in Fig. 20.

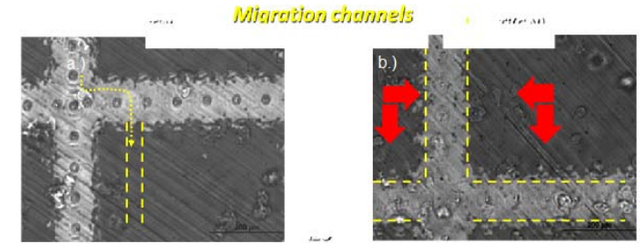

Fig. 20. Surface fictionalization influence on the cell migration and proliferation a.) Cell migration along the channels which were formed in the titanium nitride layer b.) cell migration along the channels formed in the titanium carbonitride layer

It was found that the surface modification influenced the cell behavior. Several points were considered in order of the cell migration. The chemical composition of the coatings, surface modification, additionnal wells formation. It was visible the channels formation on the cell migration. For titanium nitride, cells migrate along the channels, surround the wells and escape through the gate

(Fig 20 a). Quite different cell- material interaction was observed for titanium carbonitride (Ti(C,N)) (Fig. 20 b). Most of the cells went into the channels, probably based on. It seems that the laser ablation changed the physicochemical properties of the coating.

2.3.2. Porous materials for tissue scaffold

The third solution which was considered in our work for the artificial small vascular prostheses. The most advanced and versatile technique for fabrication of scaffolds (support for tissue) is the electrospinning process, where large diameter prostheses used in clinics are knitted, woven or expanded today (Fig. 21). The electrospinning allows to optimize control of microstructure (flat scaffolds with inherent 3D structures and macroscopic 3D shapes (tubes)) and fiber diameters in the monadic micron range and below, all mimicking the structural properties of the biological extracellular matrix as well. The excellent mechanical properties of electrospun fibers impose micro-stress necessary for maintaining the right cell phenotype.

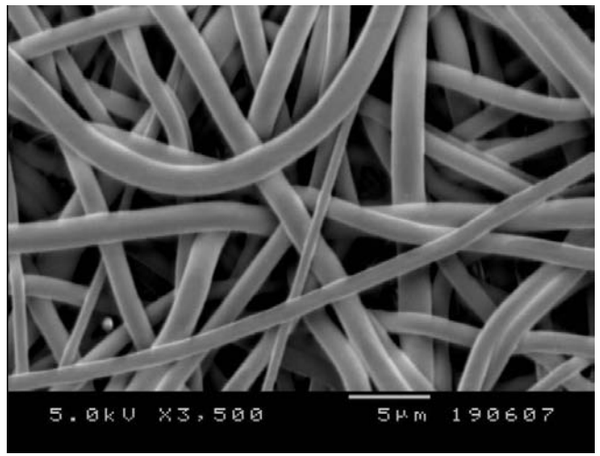

Fig. 21. Materials delivered from the Center for Biomedical Engineering and Physics; Medical University Vienna AKH Austria

2.3.3. Proteins adsorption

Protein adsorption on solid surfaces is a widespread phenomenon of large biological and biotechnological significance. Conformational changes are likely to accompany protein adsorption, but they are difficult to evidence directly. Nevertheless, they have important consequences, since the partial unfolding of protein domains can expose hitherto hidden amino acids. This remodeling of protein surface can trigger the activation of molecular

complexes such as the blood coagulation cascade or the innate immune complement system.

In the case of extracellular matrix, it can also change the way cells interact with the material surfaces and results in modified cell behavior. In fact, cells do not bind the substrate directly. They are caught by the layer of the adsorbed proteins in biological tissues. Cells are attached to each other and to the extracellular matrix, a complex but precisely defined network of proteins and polysaccharides secreted by the same or other cells is built. The type and the nature of the proteins has a significant influence on the cellular adhesion. The idea of the surface functionalization should give the possibilities to bind the specific proteins and to prepare the surface for the cellular colonization. The Fig. 22 presents the layout of the experiment. There were analyzed 6 different coatings and the porous materials. The results of the protein adsorption are presented in Table 2 and Fig. 23.

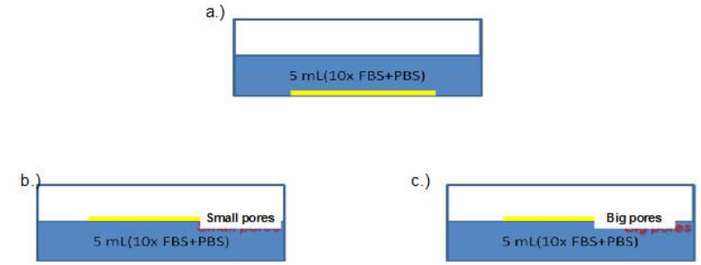

Fig. 22. Protein- material interaction experiment-
a.) layout for polyurethane (PU), titanium (Ti), titanium oxide (TiOx), silicon doped diamond like carbon (Si DLC), titanium doped diamond like carbon (Ti DLC) titanium nitride (TiN), porous material (Por);
b.) layout for porous materials with small pores;
c.) layout for porous materials with big pores

Table 2. Protein adsorption to the examined materials

Treatment	m (μg)
PU	4.08
Ti	35.13
TiOx	78.33
SiDLC	116.30
TiDLC	119.72
Ti(C,N)	134.72
TiN	139.18
Por	679.77

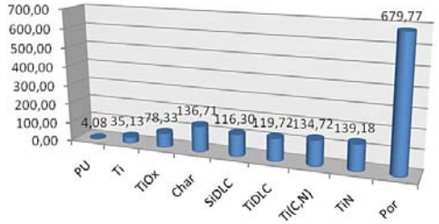

Fig. 23. Protein- material interaction experiment- results for polyurethane (PU), titanium (Ti), titanium oxide (TiOx), silicon doped diamond like carbon (Si+DLC), titanium doped diamond like carbon (Ti+DLC) titanium nitride (TiN), porous material (Por)

In most experiments, biologists try to reconstitute the cell micro-environment on plastic or glass surfaces. It is indeed well known that many cells spontaneously die (by apoptosis) when either a solid support or a suitable biochemical environment (presence of growth factors) is lacking. The cultured cells are not in direct contact with the solid surface, but instead bind specific molecular motives on the extracellular matrix adsorbed on the material surface. It is not surprising that the protein adsorption was the highest for the porous material. The most important conclusion from the presented result is that there is a new opportunity to have the gentle influence on the porous structure properties in order of the protein adsorption.

The other aspect dealing with the protein adsorption to the porous materials was associated with the pore size. "Lumina side"- small pores, "Adventita side"- big pores. The results of the protein adsorption depending on the pore size are presented in Table 3 and Fig. 24.

Table 3. Protein adsorption to porous materials

Treatment	m (μg)
Adv	160.97
Adv	177.44
Lumina	118.42
Lumina	109.02

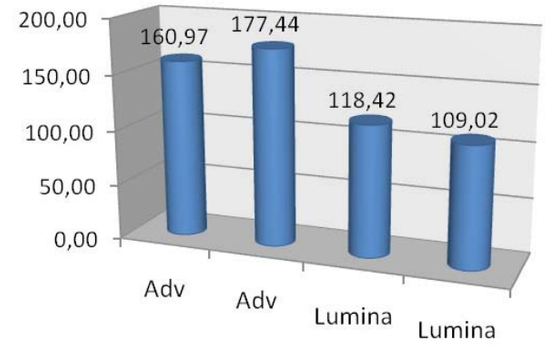

Fig. 24. Protein- material interaction experiment- results for porous materials depending on the pore size (Por)

The extracellular matrix (ECM)-like biomimetic surface modification of cardiovascular implants is a promising method for improving hemocompatibility. In the present work, poly-L lysine and hyaluronic acid multilayers were formed on polyurethane using a layer-by-layer (LBL) self-assembly technique.

The main intension of all presented examinations dealing with the cell detachment, cell spreading and the cell surviving was done to prepare the optimal surface for the tissue analogues or tissue engineered vascular grafts.

The alternating dipping of a charged surface into a poly-anion and then into a poly-cation solution usually leads to the progressive formation of films defined as polyelectrolyte multilayers (Fig. 25) [26].

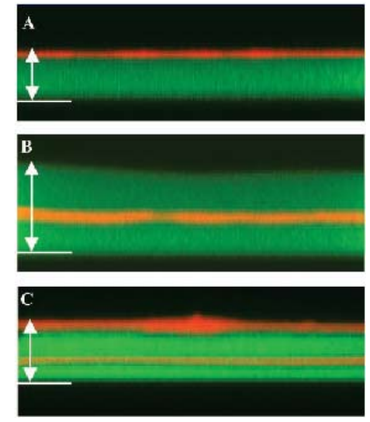

Fig. 25. Vertical sections through different film architectures containing labeled polyelectrolytes [26]

This electrostatic self-assembly method has been developed recently as a way for producing organic and hybrid organic-inorganic supramolecular assemblies without requiring extensive equipment. In particular in biomedical fields, these multilayers constitute versatile tools for the design of thin films containing macromolecules such as proteins, nucleic acids, or polypeptides with targeted properties. More information describing layer-by-layer technique is given in literature [26-29].

For the final tests of the surface functionalization, two main groups of materials were selected. Titanium carbonitride Ti(C,N) and silicon doped diamond like carbon (Si+DLC). Results are presented in Table 4.

Table 4. Protein adsorption differences between the functional and not functional surfaces

Material not modiefied	m (μg)	Material modified (functional surface)	m (μg)
PU	37.61	PU	62.17
TiCN	41.62	TiCN	87.24
Si DLC	51.18	Si DLC	53.38

2.3.4. Endothelialisation

Human Umbilical Vein Endothelial Cells (HUVEC) are isolated from a normal human umbilical vein. These cell systems are commonly used for physiological and pharmacological investigations [30-34]. The cells were deposited on the surface of the selected materials (titanium nitride, titanium carbonitride and silicon doped diamond like carbon). Figs. 26-28 and Table 5 present the survival/growth results after investigations of the HUVEC endothelium cells deposited on titanium carbonitride surface (Fig 26 a) and the situation one week after (Fig 26 b).

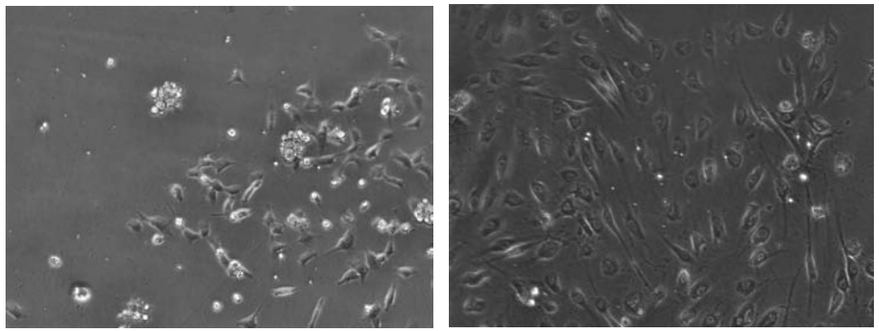

Fig. 26. HUVEC endothelium cells deposited on the titanium carbonitride surface a.) just after deposition b.) one week after

Fig. 27 illustrates the measurement done in differential interference contrasts.

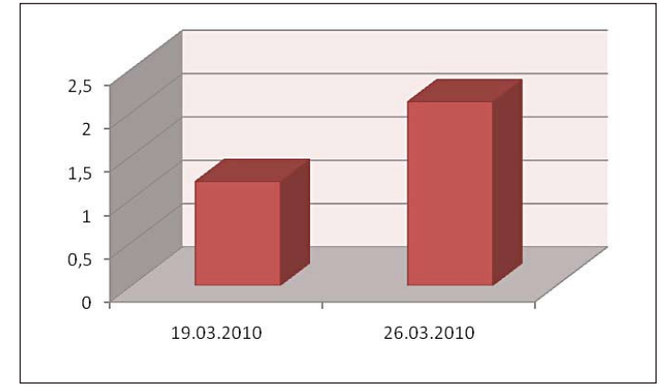

Fig. 27. HUVEC endothelium cells deposited on the titanium carbonitride surface- amount of cells as a function of the observation time

Cells were fixed and marked for immunostaining of F-actin and the nuclei. The fluorescent investigations were performed using a scanning confocal laser microscopy. One of the most typical pattern which is formed during the cell confluence is the hexagon-like. The results are exhibited in Fig. 28.

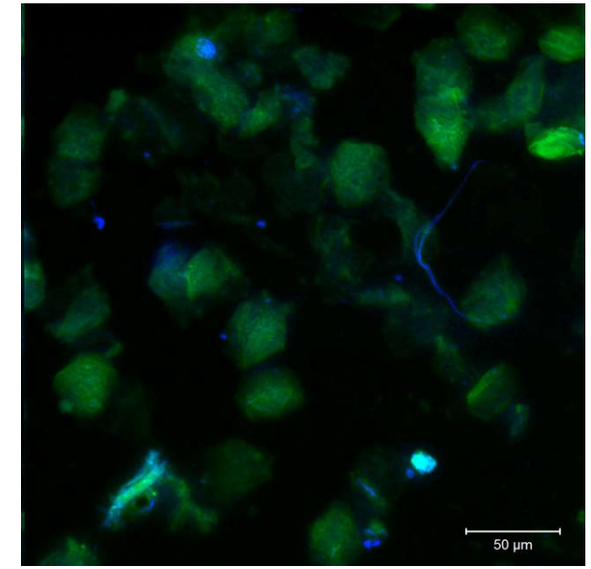

Fig. 28. HUVEC endothelium cells deposited on the titanium carbonitride surface- fluorescent observation, confocal microscope (CLSM)

Table 5. Endothelium cells growth and survival depending on the substrate material

Material	Survival	Growth
PU	+	-
PU+TiOx	-	-
PU+SiDLC	+	+
PU+Ti(C,N)	+	+
Porous	+	+

3. Conclusions

- Differences were found between the coatings according the ability of the platelet adhesion. For Ti, TiOx i DLC the highest platelets use was found. For Ti and DLC also platelets aggregates are adhering, their decreased number and small activation parameters PAC1 i SelP. TiN, TiCN i SiDLC caused the smallest platelets degradation.
- Concerning the amount of platelet aggregate, the number and the platelet activation - the best biocompatibility was found for TiCN i Si+DLC. The coating Si+DLC gives less platelet- granulocytes aggregate.
- One of the most crucial problem associated with the bioprostheses is to place the structure in the suffered areas; the environment in such regions is changed, the cellular nutrition and the gas processes are disturbed.
- One of the challenging solutions is the surface functionalization for protein adsorption and cell proliferation.

Acknowledgments

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4. Literature

1. T. M. Zielonka Angiogenesis Part I, Alergia, Astma, Immunologia, 8(4), 169-174(2003)
2. B. Major; Ablacja i osadzanie laserem impulsowym; Wydawnictwo Naukowo-Techniczne AKAPIT, Kraków, (2002)
3. J. M. Lackner; Industrially- scaled hybrid Pulsed Laser Deposition at Room Temperature; Published by Orekop sc., Kraków, Poland (2005)
4. J. Venables,. Introduction to Surface and Thin Film Processes: Cambridge University Press. ISBN 0-521-62460-6, (2000)
5. A. Pimpinelli, J. Villain; Physics of Crystal Growth: Cambridge University Press. ISBN 0-521-55198-6 (1998)
6. K Oura,.; V.G. Lifshits, A.A. Saranin, A.V. Zotov, and M. Katayama, Surface Science: An Introduction, Berlin: Springer, ISBN 3-540-00545-5 (2003)
7. D.J Eaglesham, M. Cerullo, Dislocation-free Stranski-Krastanow growth of Ge on Si(100), Physical Review Letters, 64 (16): 1943–1946 (1990)
8. N. Ivan. Stranski and L. Von Krastanow, Abhandlungen der Mathematisch-Naturwissenschaftlichen Klasse, Akademie der Wissenschaften und der Literatur in Mainz, 146, 797 (1939)
9. E. Bauer, Phaenomenologische Theorie der Kristallabscheidung an Oberflaechen I, Zeitschrift für Kristallographie, 110: 372–394 (1958)
10. I. V. Markov, Crystal Growth for Beginners: Fundamentals of Nucleation, Crystal Growth, and Epitaxy, Singapore, World Scientific, ISBN 981-02-1531-2 (1995)
11. R.V.Kukta,; L.B. Freund, Minimum energy configuration of epitaxial material clusters on a lattice-mismatched substrate, Journal of the Mechanics and Physics of Solids 45 (11-12): 1835–1860 (1997)
12. J. Venables, G. D. T. Spiller and M. Hanbucken, Nucleation and growth of thin films, Reports on Progress in Physics, 47 (4): 399–459 (April 1984)
13. Y.R.Li, Z. Liang, Y. Zhang, J. Zhu, S.W. Jiang and X.H. Wei, Growth modes transition induced by strain relaxation in epitaxial MgO thin films on $SrTiO_3$ (001) substrates, Thin Solid Films, 489 (1-2): 245–250 (October 2005)
14. C Chiu, Z. Huang, and C. T. Poh, Formation of Nanostructures by the Activated Stranski-Krastanow Transition Method, Physical Review Letters, 93 (13): 36105 (2004)
15. T.Schwarz-Selinger, Y. L. Foo, David G. Cahill, and J. E. Greene, Surface mass transport and island nucleation during growth of Ge on laser textured Si(001), Physical Review B, 53 (12): 125317 (2002)
16. G.Bauer, F. Schäffler, Self-assembled Si and SiGe nanostructures: New growth concepts and structural analysis, Physica Status Solidi (a),203 (14): 3496–3505 (November 2006)
17. O.E.Shklyaev, M. J. Beck, M. Asta, M. J. Miksis, and P. W. Voorhees, Role of Strain-Dependent Surface Energies in Ge/Si(100) Island Formation, Physical Review Letters, 94 (17): 176102 (2005)
18. F.Watanabe, G. David Cahill, and J. E. Greene, Roughening Rates of Strained-Layer Instabilities, Physical Review Letters, 94 (6): 066101 (2005)
19. Nb. : H 007301 Impact-R User Manual Version : 1.1 (2004)
20. Impact-R User Manual Version : 2.2 Nb. : H 009050 (2004)
21. B. Shenkman, A. Inbal, I. Tamarin, A. Lubetsky, N. Savion and D. Varon, Diagnosis of thrombotic thrombocytopenic purpura based on modulation by patient plasma of normal platelet adhesion under flow condition, British Journal of Haematology, 120, 597–604, (2003)

22. X. Wang, R. T. Dorsam, A. Lauver, H. Wang, F. A. Barbera, S. Gibbs, D. Varon, N. Savion, S. M. Friedman, and G. Z. Feurstein, Comparative Analysis of Various Platelet Glycoprotein IIb/IIIa Antagonists on Shear-Induced Platelet Activation and Adhesion; 0022-3565/02/3033-1114–1120$7.00 THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS Vol. 303, No. 3 Copyright © 2002 by The American Society for Pharmacology and Experimental Therapeutics, 38513/1026547 JPET 303:1114–1120, (2002)
23. C. Fallgren, A. Ljungh, B. Shenkman, D. Varon, N. Savion, Venous shear stress enhances platelet mediated staphylococcal adhesion to artificial and damaged biological surfaces, Biomaterials, 23 4581–4589 (2002)
24. M. Gawaz, Role of platelets in coronary thrombosis and reperfusion of ischemic myocardium, Cardiovascular Research, 61, 498–511(2004)
25. J. Marczak; Laserowe oczyszczanie dzieł sztuki, Inżynieria Materiałowa 6 (2008)
26. C. Picart, J. Mutterer, L. Richert, Y. Luo, G. D. Prestwich, P. Schaaf, J.C. Voegel, and P. Lavalle, Molecular basis for the explanation of the exponential growth of polyelectrolyte multilayers; PNAS October 1, vol. 99, no. 20,12531–12535 (2002)
27. G.Decher, J. D. Hong, J.Schmitt, Thin Solid Films, 210, 831–835 (1992)
28. G. B.Sukhorukov, H.Mohwald, G. Decher, & Y. M. Lvov, Thin Solid Films, 284–285, 220–223 (1996)
29. J.Chluba, J. C.Voegel, G. Decher, P.Erbacher, P. Schaaf, & J. Ogier, Biomacromolecules, 2, 800–805 (2001).
30. B. M. Leung, M. V. Sefton, A Modular Tissue Engineering Construct Containing Smooth Muscle Cells and Endothelial Cells, Annals of Biomedical Engineering, Vol. 35, No. 12, December p. 2039–2049 (2007)
31. T. Sakamoto, C. Spee, Z. Scuric, E. M. Gordon, D. R. Hinton, W. F. Anderson S. J. Ryan, Ability of retroviral transduction to modify the angiogenic characteristics of RPE cellsGraefe, Arch Clin Exp Ophthalmol., 236:220-229. Springer-Verlag (1998)
32. A. Armulik, A. Abramsson, and C. Betsholtz, Endothelial/pericyte interactions. Circ. Res., 97:512–523, (2005)
33. C. H. Arts, J. D. Blankensteijn, G. J. Heijnen-Snyder,H. J. Verhagen, P. P. Hedeman Joosten, J. J. Sixma, B. C.Eikelboom, and P. G. de Groot, Reduction of non-endothelial cell contamination of microvascular endothelial cell seeded grafts decreases thrombogenicity and intimal hyperplasia, Eur. J. Vasc. Endovasc. Surg. 23:404–412, (2002)
34. O. Ayalon, H. Sabanai, M. G. Lampugnani, E. Dejana, and B. Geiger, Spatial and temporal relationships between cadherins and PECAM-1 in cell-cell junctions of human endothelial cells, J. Cell Biol. 126:247–258, (1994)

Biological activity of chemical modified and non- modified nanodiamond particles in biomedical application

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Introduction

Diamond nanoparticles (DNP) are very promising for prosthetic implants coating and in many other applications including gene delivery to target cells [1].

The discovery of diamond synthesis under static high pressures at the end of the 1950s stimulated studies aimed at determining the application of explosion energy in diamond synthesis. For the first time, diamond was detected in a shock –compressed graphite sample in the USA in 1961 by P.J. De Carli and A.C. Jamisson [2]. V.V. Danilenko proposed and implemented (in 1962) ampoule-free synthesis with explosions in the explosion chamber instead of ampoule synthesis [3,4].

Nanodiamonds on account of their mechanical, electrical, biological properties are making a profit more and more strong interest in biomedical applications [5-9]. At present examinations are assigned to acquiring new properties of nanoparticles through their chemical modification which is creating covalent bond with organic moieties. Comparison of different functional groups obtained nanodiamonds depending on their origin causes their different behaviour in biological environment [10].Therefore examining functional groups of carbon powders coming from technological various processes, also will be recommended of the ones commercially available, next modifying through Fenton treatment[11], showing changes of functional groups after the reaction and of changes created after the application in the living cell.

The study of DNP (diamond nanoparticles) in contact with water and water solutions showed that chemical modification of detonation nanodiamond produces on its surface oxygen – containing groups with acidic character. Detonation nanodiamond forms at the front of a detonation wave after detonation of a powerful explosives mixture (TNT+hexogene) with negative oxygen balance [12].

Evaluation of their biocompatibility is of primary importance for the perspectives of their use. In this study, we evaluated the effect of prolonged contact with DNP on the antioxidant defense of human endothelial line cells (HUVEC-ST), comparing powders produced by various methods and DNP modified by the Fenton treatment to introduce surface –OH groups.

The objective of experiments was to evaluate biocompatibility of diamond nanoparticles by examining morphological status of development and angiogenic response (chorioallantoic membrane - CAM assay) in experiment performed *in ovo*, on chicken embryos' model. Nanodiamond particles did not influenced homeostasis of embryos development according to Hamburger and Hamilton standards, including detailed morphological evaluation and weight of dissected organs. Nanoparticles of diamond did not stimulate angiogenesis, new vessels developing radially toward the implant were not observed, moreover, there was a tendency to reduce vasoproliferative process. Furthermore, affinity to penetrate inside vessels was noticed, and a part of nanoparticles was seen as a tin gray stream flowing with blood in vessels of embryos receiving 5000 ppm nanodiamond particles.
Approximately one gram samples of muscle tissue from chicken embryos were excised quickly after euthanasia and stored in RNAlater TM at -20 C until isolation of RNA.
Daily skin care is the incessant challenge undertaken everyday. Together with aging it becomes higher and higher. Everyone wants to keep healthy and fine appearance of the skin. Demand of the pharmaceutical – cosmetic industry is still changing. Sometime ago people fascinated in cosmetic products containing gold or silver, however they were already used in antiquity [13].
Presently on the pharmaceutical and medical market prevail preparations containing in their chemical composition nanoparticles. Due to such a small size of the particles the biggest active surface and wide possibilities of absorbance of active substances are obtained [14].

1.1 CHEMICAL MODIFICATION METHODS

Modification ND with use Fenton reaction [11].
ND (0.25 g; detonation nanodiamond) were added to 150 mL flask with strong magnetic stirring (Teflon cover magnetic bar). Solution sulfuric iron (II) 12 g in 50 mL distilled water (degassed with argon at room temperature) was added to reaction flask. Cold solution sulfuric acid (30 mL; 96 %) was slowly added to reaction with ND (by drops). After 15 min the mixture was cooled to +5 ^{O}C and hydrogen peroxide (25 mL; 30 % solution in water) was slowly added (about 20 min) to reaction mixture with dispersion of ND (fast stirring).

After 30 min the mixture was heating to room temperature (22 ^{O}C) and keep for 90 min at temp. 30 ^{O}C (sonication 4 times 5 min).

To cold water (5 ^{O}C; 150 mL) was added the reaction mixture and start to fast filtration (with use Ace filter) on vacuum line (with water pomp). Wet modified HO-ND was washed with distilled water 10 times (100 mL). Wet grey-black solid was suspended in distilled water (60 mL) and added 0.6 g EDTA; after fast stirring (total 30 min.) and sonication can be repeated (2 times 5 min.) and start with fast filtration and washing with distilled water (10 times ; 100 mL).

Product HO-ND was dried on exicator over concentrate sulfuric acid (20 h) and on vacuum line (0.1 mm Hg; 40 oC; 10 h) – obtained 0.19 g HO-ND.

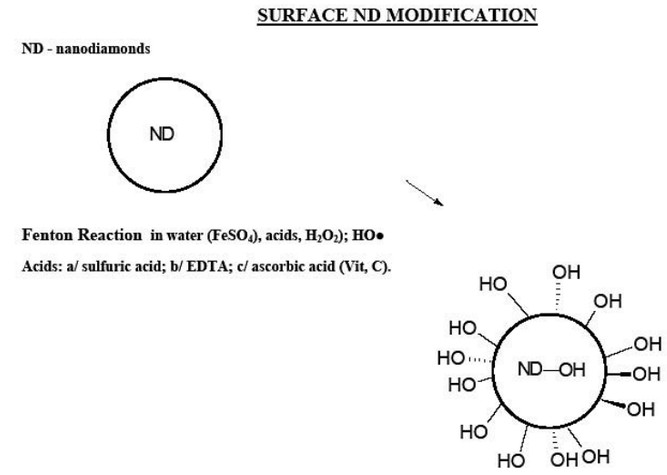

Fig.1 Surface chemical modification of nanodiamond particles [2].

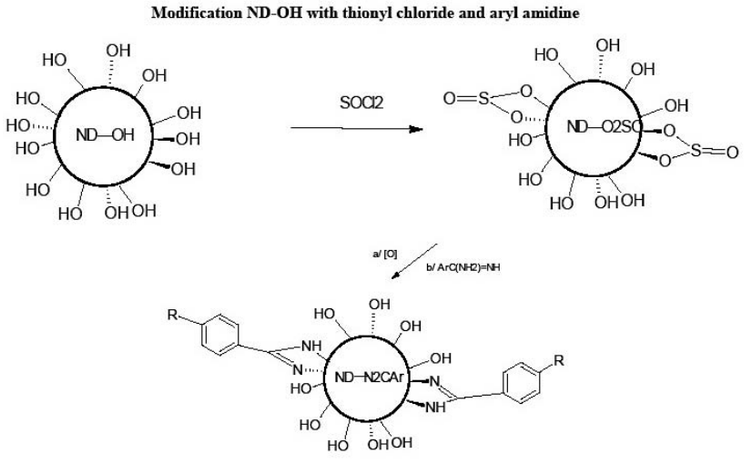

Fig.2 Modification ND-OH with thionyl chloride and aryl amidine.

1.2. Biological methods

1.2.1 Cell culture

Human HUVEC-ST cells were treated for 24, 48 and 72 h with DNP (2, 20 and 100 μg/ml) in a culture medium. The cellular level of glutathione, total antioxidant capacity of cell extracts and activities of main antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase and glutathione S-transferase) were estimated in control and DNP-treated cells.

HUVEC-ST, human endothelial cells immortalized by transfection with both SV40 large/small T antigens and the catalytic subunit of human telomerase. The cells were grown as a monolayer in medium Opti-MEM (Ivitrogen) with 2% fetal bovine serum and antibiotics: 50 μg/ml streptomycin and 10 U/ml penicillin, in an atmosphere of 5% CO2 at 37°C.

Cytotoxicity test

Sensitivity of HUVEC-ST cells to nanopowders was performed in 96-well microtiter plates by using the standard MTT colorimetric method based on the ability of the mitochondrial dehydrogenase of metabolically viable cells to reduce the tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to blue formazan product. Cells were seeded on 96-well plates at a density of 3000 cells per well and after 24 h nanopowders were added to the growth medium in a concentration range of 2-100 μg/ml. After 72 h the medium was removed, the cells washed twice with HBSS and incubated with 200μl MTT solution in growth medium (0.5 mg/ml) at 37°C for next 2 hr. The formazan crystals were dissolved with 100 μl of DMSO and the plate was read at 570 nm. The absorbance of the control cells was assumed as 100%[15].

Determination of reactive oxygen/nitrogen species production

Measurement of intracellular ROS and RNS production in human endothelial cells seeded 96-well plates (3000 cells per well) and incubated for 24 h. After that time, diamond powder were added at appropriate concentrations and the incubation was continued for another 24 h, 48 h and 72 h at 37^{O}C and 5% CO_2. After 24 h, 48 h and 72 h the monolayers of cells were rinsed in HBSS buffer with 1% albumin. Then changes was recorded by monitoring in the fluorescence of 5 mM 2',7'-dichlorodihydrofluorescein diacetate (H_2DCF-DA) and (3-amino-4-aminomethyl 2'7'difluorescein diacetate) (DAF-FM-DA). Immediately after 0,5 h, 1 h and 2 h incubation at 37^{O}C and 5% CO_2 the fluorescence was read at l ex =485 nm - l em = 538 nm for ROS and l ex =485 nm - l em = 510 nm for RNS. Plates with cells were measured after incubation in the dark.

1.2.2 The catalase assay

24 h after seeding on 6-well plates (50 000 cells per well) and growth at 37°C the cell monolayers were treated in medium with nanopowders (UDD, UDD-OH, GRAF and GRAF-OH) at concentrations of 2 μg/ml, 20 μg/ml, 50 μg/ml and100 μg/ml for 24, 48 and 72 hours. After incubation cells were washed three times with HBSS. Afterwards the cells were trypsynised, washed HBSS (3000rpm, 23°C, 5 min.) and the cell pellets were finally resuspended in 200 μl of PBS and probes were frozen at -20°C. The total extract was centrifuged at 300xg for 15 min at 4°C. Aliquots of supernatant were used for enzyme assay.
The CAT activity was assayed by monitoring the disappearance of H_2O_2 at 240 nm. The catalase activity was calculated in terms of U/mg protein, where one unit is the amount of enzyme that catalyzed the conversion of 1μmole H_2O_2 in a minute [16].

1.2.3 Glutathione assay

Cells seeded on 96-well black plates at the density of 3000 per well were grown at 37°C for 24 h, treated with nanopowders (2 - 100 μg/ml) in fresh growth medium and grown for the next 24, 48 or 72 h. Than the medium was removed

by gentle aspiration, the cell monolayers were washed three times with HBSS, added 60μl lysis buffer and plates were frozen at -20°C.
The GSH level in cellular lysate was deterimned by o-phthaladehyde assay and was calculated as nmoles/mg protein [17].

1.3 Chicken embryo model

Fertilized eggs (n=150, 58±2 g) from Ross Line 308 were obtained from certificated hatchery, Dembowka, Poland and stored during 4 days at 12 °C. The eggs were weighed and randomly divided into 5 groups, each with 30 eggs; group I (control) – no treated, group II (placebo) – PBS, group III - nano-diamond hydrosol 50 ppm, group IV – nano-diamond hydrosol 500 ppm, group V – nano-diamond hydrosol 5000 ppm. The eggs were incubated at standard conditions (temperature 37.7 °C, humidity 60 %, turn once per hour). Vascular system status and angiogenic assay were evaluated using chicken embryo chorioallantoic membrane (CAM) evaluation and the method based on the implantation of gelatin sponges on the top of growing CAM, on day 8 of incubation (IP-CAM) [18].
CAM evaluation: hydrosols of nano-diamond and PBS were given in ovo by injection to air sack to 15 eggs from each group. Eggs were disinfected with 0.05 % KMnO4, solutions were injected using sterile 1ml tuberculin syringe and 26 gauge, ½ inch needle and the injection holes were sealed with sterile tape. After 12 days of incubation eggs were broken and embryos were very gently hold out on the Petri dishes and observed using stereoscopic microscope Olympus SZX10 with Imaging Software Cell.
IP-CAM evaluation: on 3 day of incubation a 1cm^2 window in the shell was opened and 3 ml of albumen was removed. The window was sealed with sterile aluminum foil and eggs were put to the incubator and incubated during 5 days. At 8 day of incubation egg shells' windows were opened and sterilized gelatin sponges loaded with experimental hydrosols and PBS were placed over the developing CAM. Then windows were sealed, eggs were put back to the incubator and incubated during 72 hours. At 11 day of incubation morphology of blood vessel formation at the area around the implant were observed using microscope Olympus SZX10 with Imaging Software Cell.

1.4 PCR method

RNA was isolated using the SV Total RNA Isolation System (Promega) according to the manufacturer's protocol. It was translated into cDNA using both random hexamer priming as well as oligo(dT), and the products of these reactions were mixed. The PCR primer oligonucleotides were designed from chicken mRNA sequences for VEGF, basic FGF, PCNA and PAX7 and PCR conditions were optimized on chicken muscle cDNA samples. The primers produced amplification of the expected band on muscle cDNA samples from control chicken embryos and of larger size on chicken genomic DNA. Quantifying of enzyme cDNA levels was subsequently done by real time PCR using SYBR Green I detection and the LightCycler System (Roche Diagnostics). For each sample, (200 ng) of cDNA template was added to 12.5 μl of (QuantiTect) SyBR green master ix (Roche) and 0.15 μl of both forward and reverse primer (final concentration of 0.6 μM each) were added to a total volume of 20 μl with RNA-free water. An initial denaturing step at 95°C for 15 min was followed by 40 cycles with a denaturing step at 94°C (15s), an annealing step at 56 C (30s) and an elongation step at 72°C (60s) As reference genes were used beta-actin, Ep1-alpha and GAPDH. For analysis, cycling reports and melting curves were evaluated. All the reactions were done in triplicate.

1.5 Studies on human patients

Tests were conducted after approval by the Bioethics Committee acting in the Institute of Occupational Medicine in Lodz them. prof. dr. Jerzy Nofer, for medical research - Resolution 11/2009 dated 06/19/2009.
As in case of the allergy tests the same three types of detonation nanodiamond was examined. For the application of carbon powders the preparations manufactured using 50 ml of the standard cream base mixed with 0.05 g of the nanodiamond powder were prepared. Chemical compostion of the cream base was as follows: carbopol Ultrez 10, propylene glycol, glycerin, paraffinum liquidum, stearic acid, glyceryl stearate, cetyl alkohol, dimethicone, triethanolamine, EDTA.
The tests were performed on a group of 20 women aged from 22 to 33 years. The study was based on the application of the cream base mixed with one type of the carbon powder, or without (control group) on the facial skin of the patient. The application was taking place two times a day (morning and evening) for 4 weeks. The influence of carbon powders and the standard cream base on the hydro-lipid skin coat was examined before the application and after 2 and 4 weeks using two types of devices:

- DermDOC, computerized, mobile device for dermal lesion medical image documentation. In macro shooting mode, an integrated LED light allows the camera directly to touch the skin and ensure the best reproduction of skin color and texture also on the photographs with the magnification of 30×. Pictures were taken on the forehead, nose cheeks and chin.
- Global Skin Analyzer MaxBeauty GSA 90.3, apparatus used for the face skin parameters determination. The principle of operation is based on a method of resistance measurement at a flow of low sinusoidal current. The following

parameters were examined: hydration, sebum secretion and pH value. Measurements were carried out at several points of the face, the same as in case of DermDoc analysis and repeated three times for each place. Next the results were averaged [14].

2. Materials examinations

2.1 SEM

The study of chemical composition, shape and size of grains was performed using Hitachi 3000 N scanning electron microscope equipped with Thermo Noran EDX X-ray microanalyzer.

2.2 Raman Spectra

Ferrari and Robertson also argue that the peaks near 1150 cm^{-1} and near 1450cm^{-1}should not be assigned to nanocrystalline diamond or other sp3 – bonded phases. They think that these peaks are assigned to transpolyacetylene segments at grain boundaries and surface, so sp2-bonded configurations [19].

2.3 FTIR

FTIR spectra demonstrate the intensity enhancement of stretch and deformation C-H bands at diminishing of C=O, and, probably, the C=C chemical bonds content [20].

2.2 HR TEM

Electron microscopy is a useful method for observing localization of some proteins in a cell on nanometer-scale direct observation of the receptor for a bioactive substance of small molecular weight by using TEM (transmissionelectron microscopy). This concept can be widely applicable for the nanometerscale-direct observation of the receptor for any small molecules [21].

2.3 XPS

Identification and quantification of surface bonds in the nanodiamond particles was performed by XPS-X-ray photoelectron spectroscopy[22]. High-resolution transmission electron microscopy – HR TEM has been widely used to study individuals clusters in detonation nanodiamond particles. Detonation nanopowder may exhibit some inhomogenity and structural non-uniformity, so HRTEM studies are often combined with other techniques, say, X – ray diffraction applied to the same type of sample in order to get more detailed information [23].

Results

Biological Results

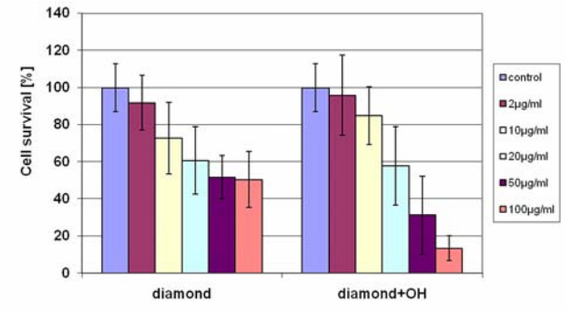

Fig. 3 Effect of nanodiamond and nanodiamond - OH on the viability by human endothelial cells.

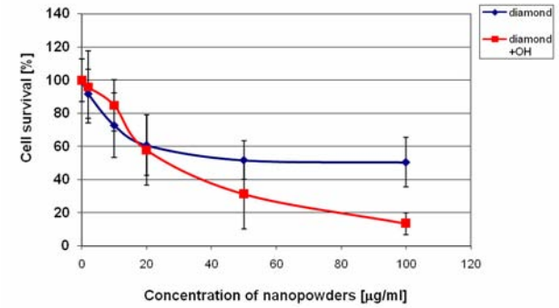

Fig.4 Effect of nanodiamond and nanodiamond - OH on the viability by human endothelial cells.

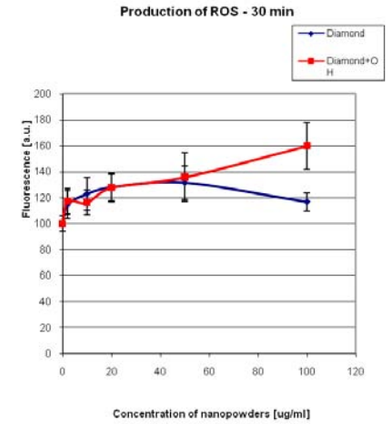

Fig.5 Production of ROS in HUVEC-ST after treatment with nanodiamond and nanodiamond - OH – 24h of incubation.

Detonation nanodiamond increases the antioxidant properties of glutathione on Human HUVEC-ST cells culture.

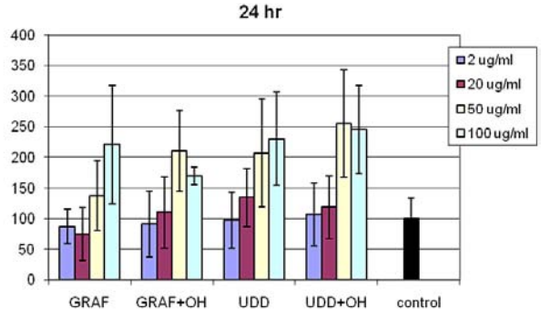

Fig. 6a The activity of glutathione in presence detonation nanodiamond in comparision with nanographite powder (control – 100%, % of control – rest results, UDD – detonation nanodiamond,GRAF – graphite after 24 h of incubation.

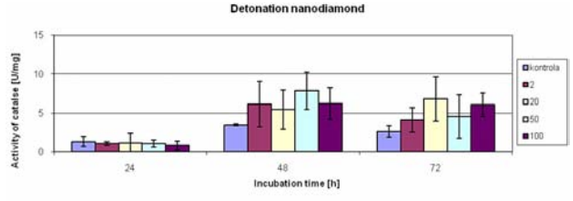

Fig.6b The activity of catalase in presence detonation nanodiamond in comparision with nanographite powder.

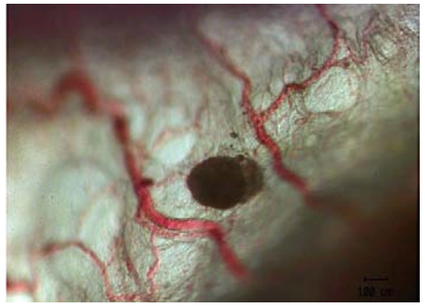

Fig.7 CAM of 12-day old chicken embryos implanted with a sponge loaded with aglomerate of nanodiamond placed onto CAM after injection of diamond nanoparticles (5000 ppm) in ovo.

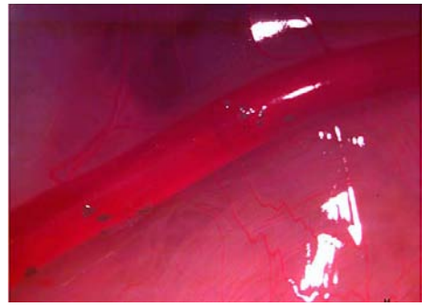

Fig.8 Distribution of diamond nanoparticles and their aglomeration in chicken embryo's CAM after injection of diamond nanoparticles hydrosol (5000 ppm) in ovo; diamond nanoparticles aglomerates located arounded vessels to force them.

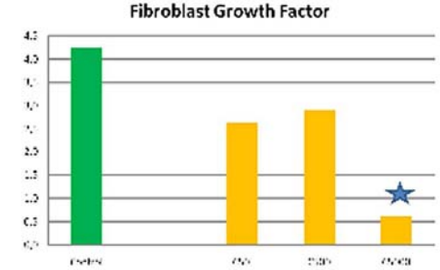

Fig. 9 The graph shows the decreasing of level of mRNA FGF in muscles by nanodiamond particles – the inhibition of angiogenesis *in vitro*.

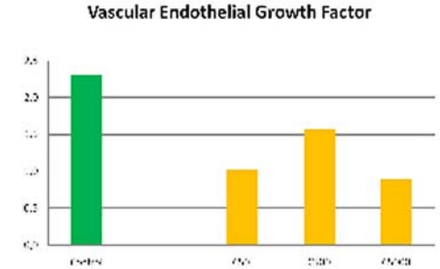

Fig.10 The graph shows the decreasing of level of mRNA VEGF in musceles by nanodiamond particles – the inhibition of angiogenesis *in vitro*.

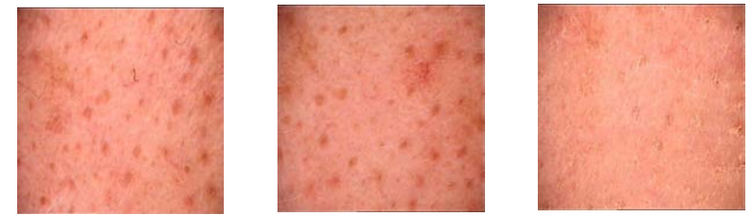

Fig. 11 are presented structures of oily and acne complexion of patient's chick before and after application of cream with detonation powder before, after 2 weeks, after 4 weeks[14].

Noticeable improvement of the hydro-lipid skin coat functioning is visible. The moths of the sebaceous and sweat glands were shrinking with time and therefore indirectly the water was retained it the organism and the sebum secretion was decreased. It is worth to notice that in case of the control group using only the standard cream base such spectacular enhancement was not observed.

Chemical Modification Results and Materials Characterization

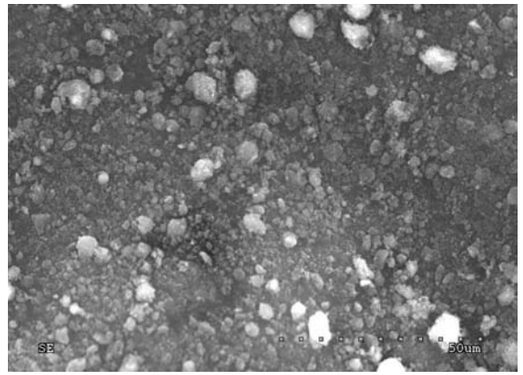

Fig. 12 SEM image of detonation nanodiamond before chemical modification [24].

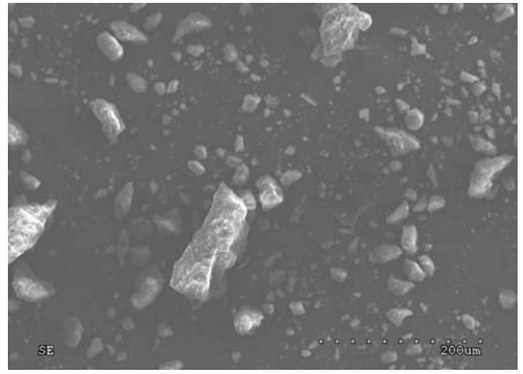

Fig. 13 SEM image of detonation nanodiamond after chemical modification [24].

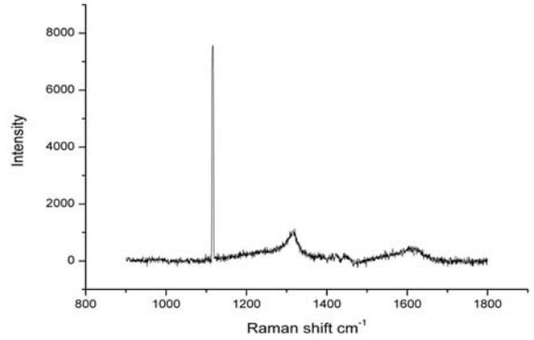

Fig.14 Raman Spectra of detonation nanodiamond before chemical modification.

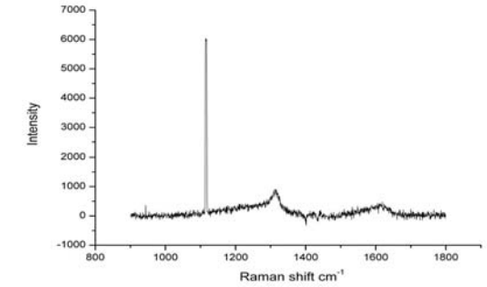

Fig. 15 Raman Spectra of detonation nanodiamond after chemical modification [24].

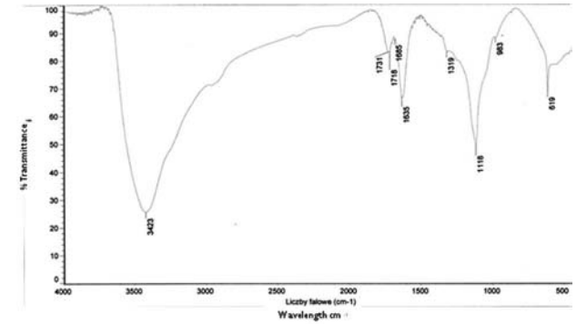

Fig. 16 FT-IR Spectroscopy of detonation nanodiamond before Fenton treatment [24].

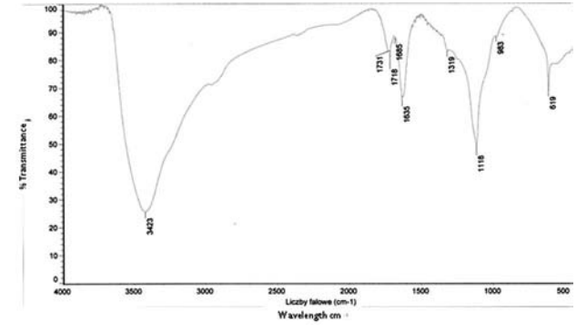

Fig. 17 FT-IR Spectroscopy of detonation nanodiamond after Fenton treatment [24].

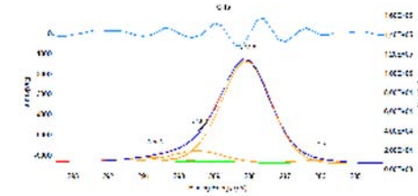

Fig. 18 XPS of nanodiamond particles manufactured by detonation method [22].

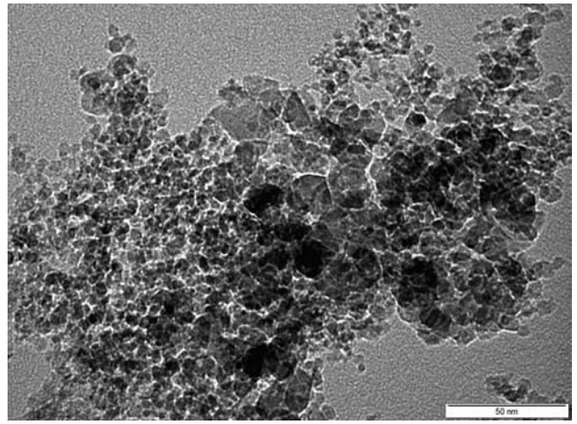

Fig. 19 HR TEM image of nanodiamond particles manufactured by detonation method [6].

Detonation nanodiamond obtained in order to Danilenko has the sherial shape size grains from 2 to 10 um, what they depict SEM images (Fig.12).
Detonation nanodiamonds after chemical modification have the smaller grain sizes they are having a tendency for creating conglomerates (Fig.13). Applying different scales of SEM images for measurements of the size of the grain is caused by the necessity of getting like the most distinct images.
Raman spectra for the samples a and c show peaks corresponding to interest us diamond bonded at 1318 cm^{-1} and peak at 1600 groups OH (hydroxyl) at 1600 cm^{-1}. (Fig.14). After fenton treatment it was observed that the carbon perecentage of pristine DNs was reduced.We interpret this decrease as a reflection of the introduction of oxygen functionalities. After fenton treatment it was observed that experienced a decrease and shift of the peak which may indicate a reduction sp^2 bonds and OH groups (Fig.15).
The types functional groups examined samples and changes in these groups introduced in the Fenton treatment were determined by FT-IR. Figure 16 shows the IR spectra of detonation nanodiamond. As it can be seen in this Figure the sample has a peak at 3423 responsible for the presence of our interest hydroxyl (OH) groups.
Figure 17 shows FTIR spectra this sample after Fenton treatment. The intensity of the band (broad) about 3421 cm^{-1} corresponding to hydroxyl groups grows and concomitant it was observed appearance of the carbon- oxygen (C-O) single bond at about 1124-1205 cm^{-1}. It is evidence of surface hydroxylation by Fenton.

Conlusions and Discussion

It has been demonstrated that depending on the origin the diamond nanoparticles are rich in various functional groups which can result in diverse behavior in biological environments. In the present work, we have shown that by Fenton treatment diamond nanoparticles to underwent covalent functionalization, *(destroy the undesirable soot that causes agglomeration of the nanoparticles).* In case diamond nanoparticles detonation powders this chemical reaction increases the population of OH groups that are the reactive sites and can be used for subsequent covalent attachment of a large variety of functional chemical compounds. Future studies will be directed at these processes.
The results provide new data demonstrating a significant biocompatibility of the DNP used which, especially at the lower concentrations, did not affect significantly the parameters of antioxidant defense measured, indicating a neglibible induction of oxidative stress.
Examinations on human patients revealed that carbon powders applied for the right kind of skin have a beneficial impact on the hydro-lipid skin coat. Detonation powder should be applied for all types of skin, especially for the dry type.
On molecular level, in vitro, in ovo and in clinical research detonation nanoparticles is very bioactive.
Results of materials experiments proved that we used detonation nanodiamond (2-10 nm grain size) which contains about 80% of sp3 phase and rest sp2 (Fig.18).

References:

1. K. Mitura, S. Mitura, in: V. Zhitomirsky and A. Raveh(eds.), Proc. The Internat. Conf. on Superhard Coatings, Kibbutz Ein-Gedi, Israel, 27 Feb.-01 March 2006, *Surface Coatings Technology*, 201 (2007) 6063.
2. P.J. De Carli, A.C. Jamisson, Science 113, 3467 (1961).
3. V.V. Danilenko, Synthesizing and sintering of diamond by explosion, Moscow: *Energoatomizdat,* 2003 (in Russian).
4. V.V. Danilenko, V.I. Trefilov, V.N.Danilenko, USSR Patent No SU 181, 329 AZ (1991)
5. K.Bakowicz, PhD Thesis, Technical University of Lodz, (2003), in Polish.
6. K.Mitura, Journal of Achievements in Materials and manufacturing Engineering, Vol. 37, (2009), 317-322.
7. K.Mitura, A.Karczemska, P.Niedzielski, J.Grabarczyk, W. Kaczorowski, P.Louda, S. Mitura International Journal of Nanomanufacturing, Vol. 2, (2008), 29-39.

8. S. Mitura, K. Mitura, P. Niedzielski, P. Louda, V. Danilenko, Journal of Achievements in Materials and manufacturing Engineering, Vol. 16, (2006), 9-16.
9. S. Mitura (ed.), S. Mitura, A. Mitura, P. Niedzielski, P. Couvrat: *Nanotechnology in Materials Science*, Elsevier, 2000.
10. W. Mark Saltzman, Cambridge University Press, United States of America, 2
11. R. Martin, P.C. Heydron, M. Alvaro, H. Garcia, *Chem. Mater.*, (2009), 21, 4505 – 451
12. B.V. Spitsyn, S.A. Denisov, N.A. Skorik, A.G. Chopurova, S.A. Parkaeva, L.D. Bel yakova, O.G. Larionov, Diamond Related Materials, (2010), 19, 123-127.
13. E. Budzisz, New look at gold complexes as potential antitumor drugs, Polish Journal of Cosmetology 2006, 9(4) 212-221.
14. M. Batory, K.Bakowicz-Mitura, Pol.J.Cosmetol., (2009), 12, (4), 238-250.
15. T. Mosmann, J Immunol Methods 1983;65:55–63.
16. H.Aebi, Methods Enzymol**, (**1984)
17. Akerboom TPM, H.Sies, Meth Enzymol, (1981); 77: 373-382.
18. D. Ribatti, A. Gualandris, A. Vacca, M. Iurlaro, L. Roncali, L. Presta, M. Presta, J. Vasc. Res. 34(6), 455 (1997).
19. A.C. Ferrari, J. Robertson, Phys. Rev. B 63 (121405), (2001), doi:10.1103/PhysRevB.63121405. (2006), 15, 296-299.
20. B.V. Spitsyn at all, Diamond and Related Materials
21. Y. Manabe, T. Sugimoto, T. Kawasaki and M. Uedaa,Tetrahedron Letters 48 (2007) 1341–1344.
22. M.Czerniak–Reczulska, P.Niedzielski, A.Balcerczyk, G.Bartosz, A. Karowicz–Bilińska, K.Mitura, Journal of Nanoscience and Nanotechnology, 10, (2010), 1-7.
23. X. Tao, X. Kang, Z. Jiazheng, Mater. Sci. Eng. B 38, L1 (1996).
24. K.Mitura, R.Woś, J.Skolimowski, Potencial and Application of Nanotreatment of Medical Surfaces, Book of Extended Abstracts, (2010), 13-16, ISBN978-80-7372-631-7.

Application of Diamond for Manufacturing Microfluidic Devices

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Abstract

Nowadays a great spectra of interest is turned to shortening the time needed for the analysis of biomolecules such as DNA or proteins. Miniaturization of standard electrophoretic techniques reduces the analysis time. Diamond microfluidic devices are small size plates with microchannels and reservoirs on the surface with geometry depending on the application. In those microstructures electrophoretic separations take place. Tested samples migrate inside microchannels under the influence of electric field and are separated depending on electrical charges and molecular masses. Electric field causes the generation of Joule heating which is a major problem connected with using of these devices. Miniaturization of the conventional electrophoresis and application of materials with high thermal conductivity will allow to minimize that phenomenon. This paper presents the technique of producing microchips made-up of polycrystalline diamond fabricated in MNT ERA–NET project [1]. In order to manufacture diamond microfluidic devices, replica method was used. The mould was fabricated using standard photolithography methods. Adixen reactor was used to obtain high aspect ratio vertical walls through plasma etching. Thick polycrystalline layer was deposited onto silicone mould with use of MPCVD (Microwave Plasma Chemical Vapor Deposition) method. Silicon mould was removed by wet etching process.

Keywords: Diamond, replica method, microfluidic device, electrophoretic chip

Introduction

Microchip technology for bio-chemical applications is developed for about twenty years. Research on Biomedical Microsystems has highly interdisciplinary nature and covers biology, chemistry, medicine, physics, fluid mechanics, materials science, engineering and many more. In the beginning of 1990-ies the microfabrication technology known from microelectronics was applied to microsystems for fast separations electrophoretic chips [2]. Such devices are also called "lab-on-a-chip", "μTAS" (micro-total-analysis systems) or microfluidic devices, because different chemical or biological processes and reactions take place inside the systems of microfluidic capillaries and microstructures manufactured on the surface of small plate. Such devices have many applications in biology, medicine, chemistry, ecology and many more. They can be used for biomolecules electrophoretic separations, medical diagnosis, and for instance for space trips astrobiology purposes. The samples and different reagents can be mixed, separated, diluted and controlled using micropumps, microvalves, mixers, systems of microcapillaries and different electronic devices often included to the chip.

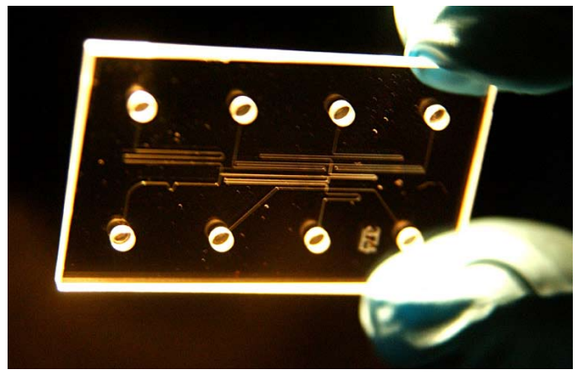
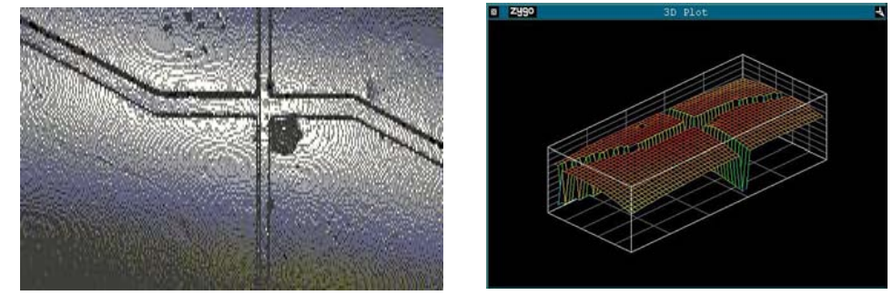


Fig.1. Example microfluidic device from NASA website: www.nasa.gov

Different applications of microfluidic devices depend on the imagination of designers. Materials used for their fabrication are commonly polymers, glass, silicon or quartz. The most common are polymers such as PDMS or PMMA, because of their low cost and simplicity of manufacturing microstructures on their surface. However, disadvantage of polymers are poor physical properties of these materials such as low thermal conductivity. So searching for different materials is necessary. Figure 2 shows the crossing microchannels of polydimethylosiloxane (PDMS) chip. Applying this material allows to manufacture cheap microdevices of desired microstructures geometries.



a) **b)**

Fig.2. Microchannels cross on polydimethylosiloxane (PDMS) microchip, microchannels 50μm wide, 100μm deep (ZYGO microscope with interferometer)

Few years ago we used for the first time monocrystalline sapphire (Al2O3) for manufacturing electrophoretic chip with 64 paralel microchannels (part of it is seen in Figure 3, in 64 microchannels 64 samples can be analysed at the same time). Monocrystalline Al2O3 has very good properties but it is difficult to shape. Microchannels have been etched with plasma, however only microchannels of about 15 μm deep were obtained.

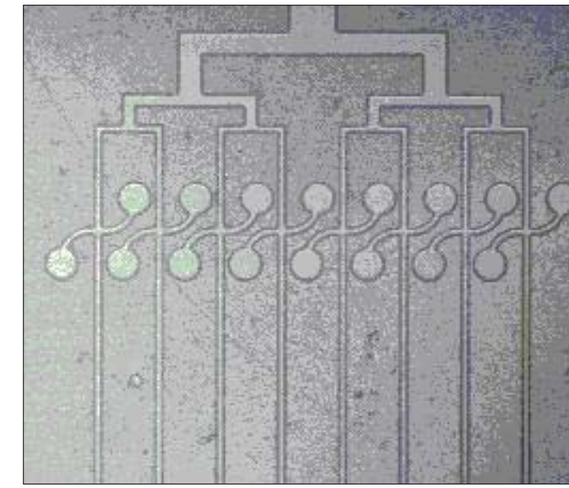
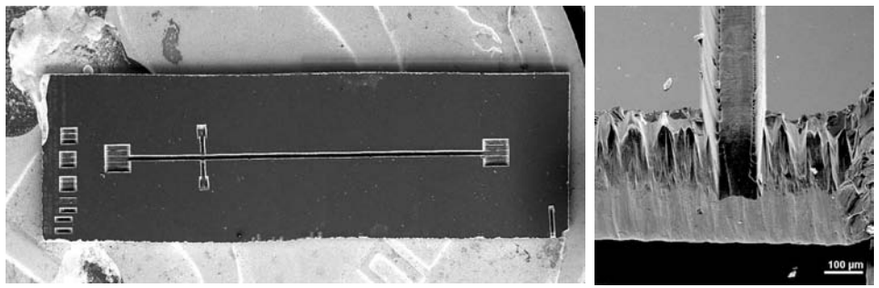


Fig.3. Sapphire (Al2O3) microchip with microchannels 75μm wide and 15 μm deep, microstructures manufactured with plasma etching

Diamond has extremely interesting properties, is opticaly transparent, chemicaly inert (so doesn't react even with very reactive reagents), biocompatible, has high electrical resistivity and the highest known thermal conductivity coefficient (2000 W/mK for monocrystalline material) [3]. It is material that

could be applied for manufacturing microfluidic devices for extremal purposes. For the moment, polycrystalline diamond material is quite expensive, however, could be much cheaper if diamond technologies will be widespreadly applied (similarly as the prices of silicon technology). The example of first diamond microchip manufactured in the year 2000 in the General Physics Institute of Russian Academy of Science, is seen in Figure 4. Problem with diamond is that it is a very difficult material to shape, it is difficult to obtain deep microstructures on its surface. Deep microchannel was obtained with laser etching. Later in this article, the replica method will be shown to obtain deep microstructures in diamond.



a) b)

Fig.4. Polycrystalline diamond microchip with microstructures manufacture with laser, microchannel length is 11 mm, depth-150μm, width-100μm

Bio-molecules (e.g. DNA) separation time becomes very important analytical parameter (laboratory effectiveness) [3-5]. Miniaturization of electrophoretic devices enables tests shortening, as well as gives reduction of bio-samples and expensive reagents volumes. Micro-chips made-up of polycrystalline diamond (at the moment standard is plastic or glass) were containing different systems of fluidic channels few tens micrometers wide, up to two hundred micrometers deep, with reservoirs at the ends. These micro-channels are filled with the buffer. Bio-samples of extremely small volumes are introduced from lateral cross-channels to the central micro-fluidic channel, where electrophoresis takes place. Sample - a mixture of chemical molecules with different formula weight and electrical charge – tends to separate under strong electrical field (few kV). Heavy bio-chemical molecules with small particle charge slowly migrate to adequate electrode placed in the reservoir at the channel end. At the same time light bio-molecules with high charges migrate faster. Migration speed depends on the mass/charge proportion of bio-molecule and electrical field. Higher electrical field means separation time shortening, but also extensive, undesired Joule heat generation. Application of diamond (high electric strength and highest-ever heat conductivity coefficient) as the microchip material, instead of plastic or glass, enables both: application of the higher electrical fields and much better heat dissipation from the micro-channel region. Diamond's other mechanical, chemical and optical parameters, as well as its biocompatibility, make it very interesting material for special applications.

Materials and methods

In order to manufacture diamond microfluidic devices, a replica method was applied (illustrated on Figure 1). Overall concept is based on the mould technique [6-7, 10-15].

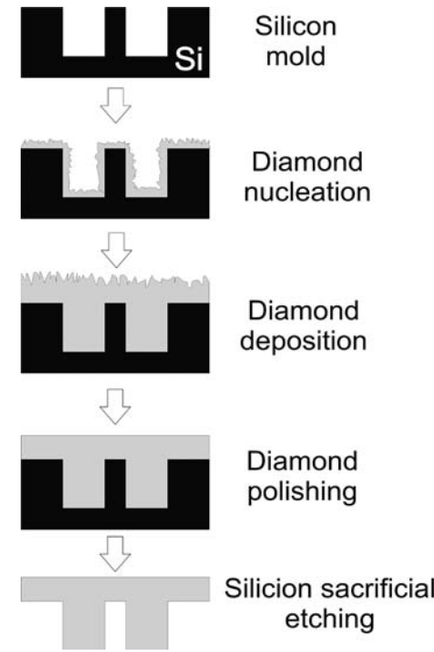


Fig.5. A process flow scheme for the manufacturing of diamond replicas

Silicon moulds were fabricated in ITE Warsaw, with use of 3 mm thick wafers (4 inch) as the mono-crystalline silicon substrates, oxidation and metal deposition steps, one level photolithography and high aspect ratio, deep plasma etching. Both sides of the silicon wafers were oxidized in the diffusion furnance, followed by the 0.5μm thick aluminum layer deposition. Standard photolithography, with the single mask was used for the pattern transfer on the photoresist layer. To etch SiO_2/Al layers were used special chemical solution.

Plasma etching was performed with use of Adixen reactor (Bosh process) to form deep trenches. Process consists following steps: silicon etching and polymer deposition on the sidewalls, as shown on Figure 2. On the Figure 3 is shown SEM image of the vertical walls.

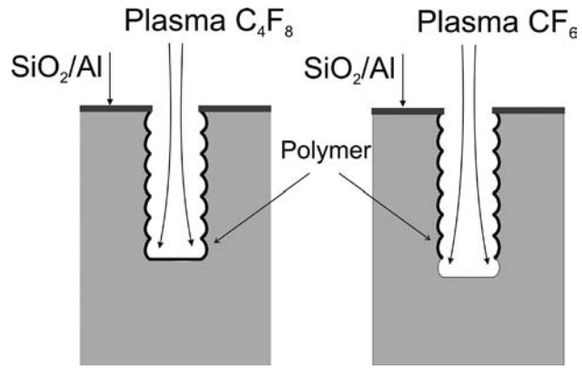


Fig.6. Illustration of the Bosch process

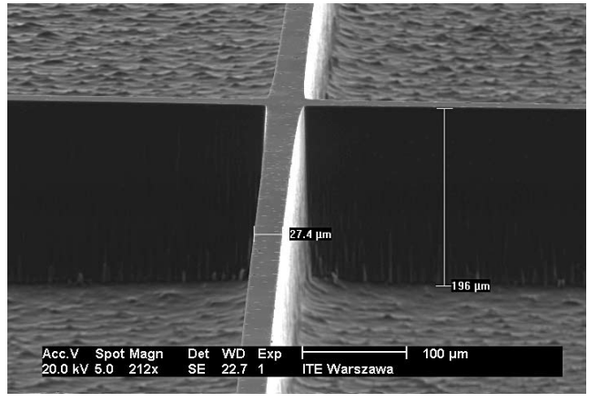


Fig.7. SEM of the silicon mould

Polymer layer on the trench sidewalls are to block plasma chemical etching. The last steps were chemically removed masking layers.

Thick polycrystalline diamond layers were deposited in General Physics Institute, Russian Academy of Science, on the silicone moulds with use of the MPCVD (Microwave Plasma Chemical Vapor Deposition) reactor system DF – 100, using CH_4/H_2 gas mixture at the following conditions: microwave power 3.6 kW, methane content 2%, total flow rate 800 sccm, pressure 87 Tr, substrate temperature 820°C. Silicon mould nucleaction was provide by bath with detonation nanodiamond disolved in ethanol [8]. Silicon mould was chemically etched-off and diamond chips were finished with the laser (wafer edges cutting and upper surface polishing).

Results and Discussions

SEM investigation was provide to demonstrate geometrical structures diamond microfluidic devices. The images with structures of microcanals was done with a Scanning Electron Microscopy Hitachi S – 3000N. Results are shown on Figure 4 and Figure 5.

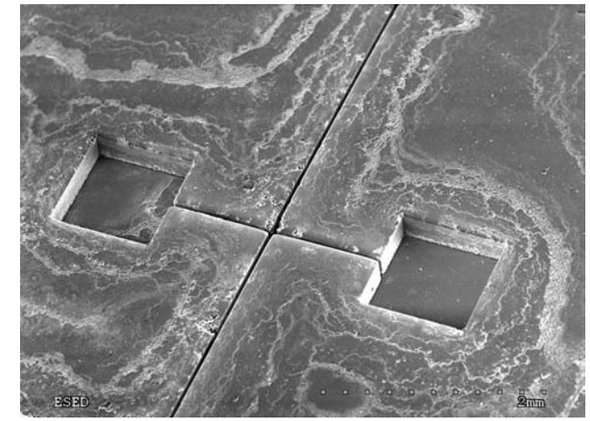


Fig.8. SEM of the fragment with two reservoirs and crossed microchannels

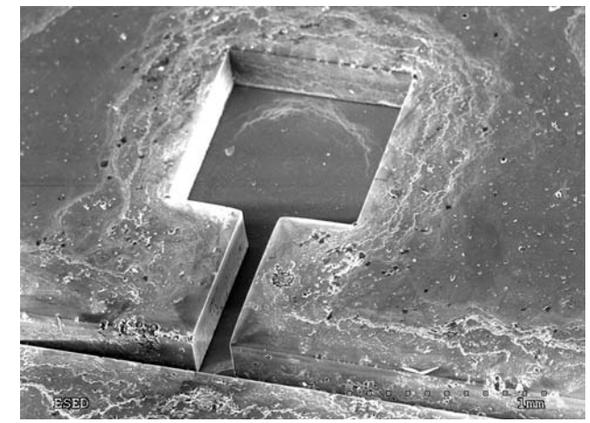


Fig.9. SEM of the fragment with reservoirs

Raman spectra were recorded with an alpha 300 RA equipped with a frequency doubled Nd:YAG laser with 532 nm excitation. The CVD sample was measured

on the area on the smooth side. The measurement was performed in the Spectral Imaging Mode with 10 μm x 10 μm and 100 x 100 pixels with an integration time of 112 ms/spectrum. With these spectra the basis analysis, a fit procedure, was calculated. Example result shows diamond spectra at position 1332 cm^{-1} without any additional peak (Figure. 6), this confirms high quality of the material [9].

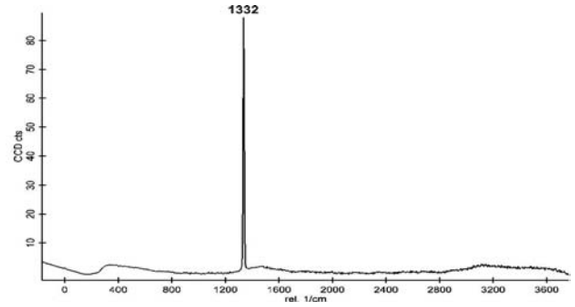


Fig.10. Raman spectra for polycrystalline diamond plate

Conclusions

Polycrystalline Diamond Microfluidic Devices, manufactured with use of replica technique have been demonstrated. Microchannels (25μm wide and 250μm deep) with good roughness and perpendicular to the surface have been fabricated. Diamond's extreme properties such as the highest known thermal conductivity, remarkable bio-adhesion properties, high electrical resistivity, optical properties, and chemical inertness, make it a great material for this application. Standard microfluidic devices are produced with glass, polymer, silicon and surface modifications of these materials. Diamond chip with microchannels network will provide extremely efficient Joule heat dissipation upon electrophoresis. In addition it will radically improve the process stability, resolution and will increase the molecular separation rate, while allowing the optical methods for the separation control. Using polycrystalline diamonds for this application allows higher voltage and therefore provides shorter times of biochemical analysis.

Acknowledgments

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References

1. "Diamond Microfluidic Devices for Genomics and Proteomics", EU project ERA-NET MNT/98/2006, 2007-2010.
2. A.Manz, D.Harrison: E.Verpoorte, J.Fettinger, A.Paulus, H.Ludi, H.Widmer, J. Chromatogr. A593 (1992) 253.
3. Karczemska A., Sokolowska A., "Materials for DNA Sequencing Chip" Journal of Wide Bandgap Materials, Vol.9, No.4, 2002 (243).
3. W.C. Tian, E. Finehout "Microfluidics for Biological Applications" Springer 2008, ISBN: 978-0-387-09479-3.
4. D. Wu, J.Qin, B. Lin "Electrophoretic separations on microfluidic chips" Journal of Chromatography A 1184 (2008) 542-559.
5. C.S. Henry "Microchip Capillary Electrophoresis Methods and Protocols" Humana Press 2006, ISBN: 1-58829-293-2.
6. Ralchenko V., Schirone L., Sotgiu G., Zakhidov A., Baughman R., Khomich A., Nunuparov M., Vlasov I., Frolov V., Karabutov A., "Direct growth of diamond components", in Proceedings of the 6th International Symposium on Diamond Materials, The Electrochemical Soc., Pennington, USA, Vol. 99-32, 2000, (72-79).
7. H.Björkman, P.Rangsten, P.Hollman, K.Hjort, "Diamond replicas from microstructured silicon masters" Sensors and Actuators 73, 1999 (24-29).
8. Ralchenko V., Vlasov I., Frolov V., Sovyk D., Karabutov A., Gogolinsky K., Yunkin V., "CVD diamond films on surfaces with intricate shape" ed. A.A.Voevodin at al., Nanostructured Thin Films and Nanodispersion Strengthened Coatings, Kluver, 2004 (209-220).
9. Bergman L., Nemanich R. J., "Raman and photoluminescence analysis of stress state and impurity distribution in diamond thin films", Journal of Applied Physics, Volume: 78 Issue:11, 1995 (6709 – 6719).
10. D.Witkowski, D.Obidowski, J.Łysko, A.Karczemska, „3D simulations of Diamond Microfluidic Devices", Inżynieria Biomateriałów (Engineering of Biomaterials), No 86 (2009), 14-16.
11. A.Karczemska, D.Witkowski, J.Lysko, Joule heating effects in capillary electrophoresis designing electrophoretic microchips International Conference VaPSE 2009, October 22 - 26, Hejnice - Liberec, Czech Republic, 2009.
12. A.Karczemska, D.Witkowski, V.Ralchenko, A.Bolshakov, D.Sovyk, J.M.Łysko, M.Fijałkowski, J.Bodzenta, J.Hassard, „Joule Heating Effects In Diamond Electrophoretic Microchips" Abstract Book of VIII Conference Microtechnology and Thermal Problems In Electronics, 28th of June – 1st of July 2009, Lodz, Poland.

14. M.Fijałkowski, A.Karczemska, J.Łysko, P.Louda, V.Ralchenko, S.Mitura, „Diamentowy mikrochip elektroforetyczny”, Elektronika-konstrukcje, technologie, zastosowania, nr 9/2009, str. 27-30.
15. D.Witkowski, J. Łysko, A.Karczemska, „Joule heating effects in capillary electrophoresis – designing electrophoretic microchips“, Journal of Achievments in Materials and Manufacturing Engineering, 37 (2009) 592-597.

A Comparison of two methods of Left Ventricular Reconstruction with the use of computer simulation for Patients with Ischemic Heart Failure.

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ABSTRACT

Chronic Ischemic Heart Failure (IHF) leads to Left Ventricular (LV) volume growth and a change of the ventricle shape from elliptical to circular. The pumping action of the heart is weakened and ejection fraction reduced. The current surgical therapeutic option in this group of patients include Ventricular Reshaping techniques based on reducing LV volume, i.e. surgical ventricular restoration, the Menicanti method (TR3ISVR™ procedure) and passive constraint device therapy (Myosplint procedure). The goal of this project was to model the damaged left ventricle and to computer analyse the influence of the mechanical properties of the Dacron patch and Myosplint system on LV Ejection Fraction, Shear Stress, Wall Shear Stress, myocardial wall and papillary muscle deformation. Finally, the two surgical approaches were compared. The Dacron patch and Myosplint pads can disturb LV wall dynamics, thus decreasing LV ejection fraction and increasing myocardial stress. Additionally, the stiff Dacron patch may cause mitral regurgitation due to the widening of the distance between papillary muscles. In order to improve LV wall dynamics, patches with a small elasticity modulus are preferred. Computer modelling of various LV reconstruction procedures will facilitate a more precise restoration of the shape of the infarcted ventricle, and thus improve the early and long term results in patients with Ischemic Heart Failure.

KEY WORDS: Ischemic Heart Failure, cardiovascular system, computer modelling, left ventricular reconstruction.

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INTRODUCTION

The most common cause of Congestive Heart Failure (CHF) is Ischemic Heart Disease (IHD) or prior myocardial infarctions. Postmyocardial damage of the left ventricle leads to its remodelling, which results in volume increase and ventricular shape change from elliptical to circular [1,2]. Left ventricular pumping action is reduced and weakened (EF<35). Increased myocardial wall stress stimulates left ventricular hypertrophy. Approximately 1/3 of patients with chronic ischaemic heart failure develop functional ischaemic mitral regurgitation (FIMR). Mitral valve dysfunction is a consequence of significant deformation of the subvalvular apparatus in the remodelled left ventricle. The displacement of papillary muscles towards the apex and laterally, the increased tenting area, and annular dilatation are the main mechanisms responsible for mitral regurgitation. Cardiac transplantation is a well know method of IHD treatment; however, as the number of transplants is limited and has decreased in the last years, alternative surgical procedures are being developed and introduced. Experimental investigations offer new treatment concepts for CHF and IHD patients. One such modern method of CHF therapy is Ventricular Reshaping, which consists in volume reduction, and involves both surgical treatment and passive constraint device therapy. According to the law of Laplace, with an increase of the left ventricular radius and cavity pressure (e.g. aortic stenosis, hypertension), the wall thickness and wall stress increase too [11,12]. TR3ISVR ™ and Myocore Myosplint represent new surgical strategies of Ventricular Reshaping in the treatment of patients with CHF. TR3ISVR ™ is a highly effective surgical therapy in the treatment of ischemic CHF and is one of the methods used in the Silesian Centre for Heart Diseases (Zabrze, Poland) since March 2003. To date, 110 such procedures have been performed, with 4.5% mortality [7] (Fig.1a). Ventricular remodelling occurred after: anterior myocardial infarction (100%), lateral myocardial infarction (5.5%), posterior myocardial infarction (6.6%), involving one wall (89%), involving one or two walls (multi-territory) (11%). Mitral regurgitation was present in 38.5 %. The complications following LV reconstruction concern diastolic dysfunction, mainly due to diastolic volume, correct shape (Fig.2) and mitral regurgitation. For this reason, we endeavoured to compare the TR3ISVR ™ and Myosplint procedures (MyocoreTM Myosplint system) with the use of computer modelling methods (Fig.1) [7].

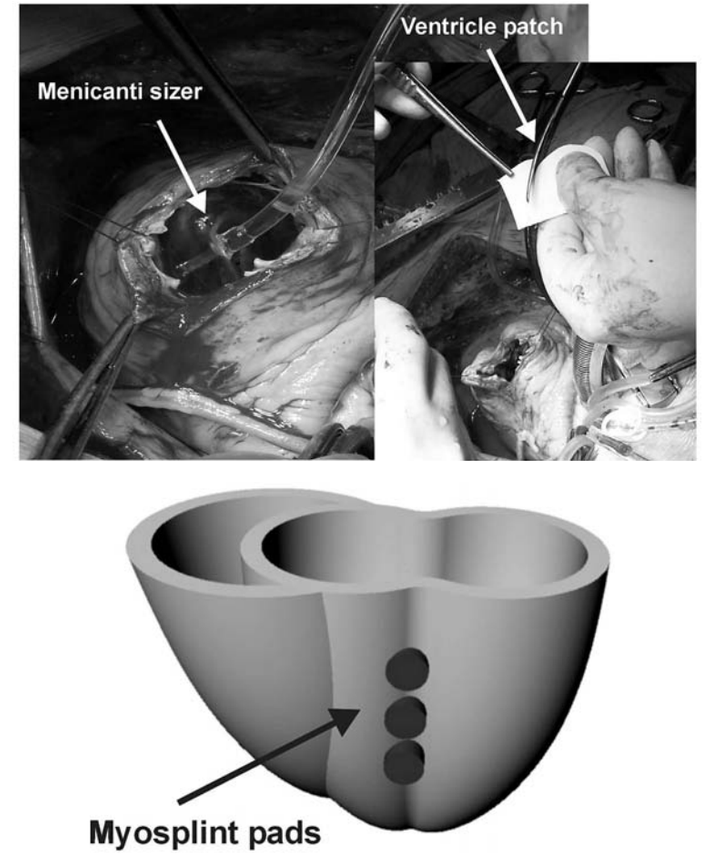

Fig.1. Two methods of LV reconstruction:
a) TR3ISVR™ (from Silesian Center for Heart Diseases),
b) Myosplint

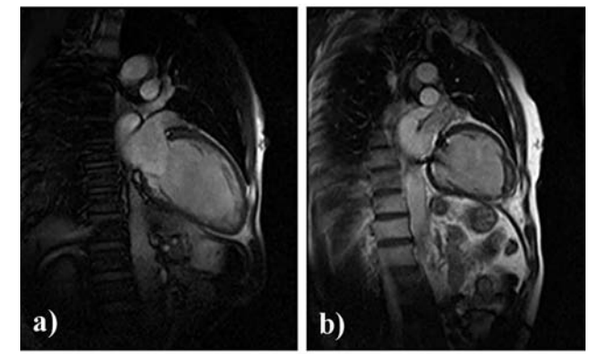

Fig.2. MRI image of LV in early systole; a) before, b) after TR³ISVR ™ procedure (from Silesian Center for Heart Diseases).

Using the TR3ISVR™ procedures, the correct shape and volume are obtained by cutting or sewing up the postinfarct myocardial segments. The LV opening is closed with a patch. In order to make the Surgical LV Reconstruction more effective, a heart resizing device (balloon-sizer Mannequin^{TM}) is inserted into the left ventricle [3, 4, 5]. The surgical aims of TR3ISVR are: apex reconstruction, optimal reduction of the ventricular volume in the anterior and septal components of the ventricle, restoration of the elliptical ventricular shape, correct mitral valve position, correct muscle fibre orientation, correct orientation of the papillary muscles towards the apex, and complete revascularization.

The Myosplint procedures (Myocore™ Myosplint system) is a passive constraint device therapy where the reduction of the LV size is obtained by two (three) pads passing through the chamber, thus creating two small chambers. The results is decreased LV wall tension according to the law of Laplace [2, 7]. The first clinical Myosplint implantations were performed as an acute feasibility study in July 1999 at the Cleveland Clinic. Until 2003, only 21 such procedures were performed. The Myosplint technique is still in its experimental phase.
The goal of this project was to computer analyse the influence of the mechanical properties of the Dacron patch and Myosplint system on LV Ejection Fraction Volume, Shear Stress, Wall Shear Stress, Deformation of the LV myocardial and papillary muscle, so as to compare the efficiency of the two CHF treatment procedures: Menicanti (TR3ISVR™) and Myosplint (Myocore Myosplint™ system).
The Silesian Centre for Heart Diseases participates in a multicentre trial: Surgical Treatment for Ischemic Heart Failure (STICH) that evaluates the clinical benefits of combined CABG+SVR vs. CABG alone.

METHODS

Based on medical image data (USG, MRI) [8], two 3D models were created using Matlab-segmentation (Fig.3). The geometry of the models is based on medical image data of a patient with anterior myocardial infarction; in order to create the FEM mesh, the model was simplified using Gambit software (Fluent Inc.). The first model shows the anatomical heart including mitral and aortic valves, whilst the other shows a simple ellipsoidal LV model.

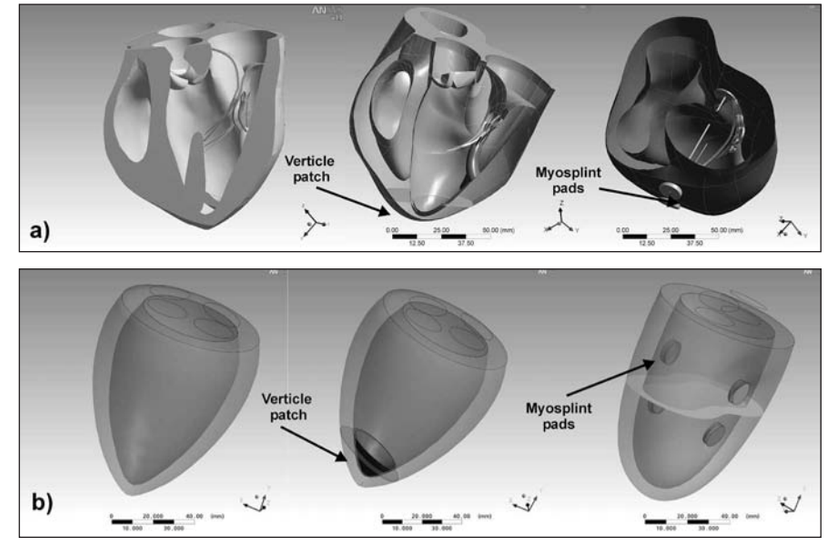

Fig.3. The Computer models: a) of the heart b) of the simple FSI models of LV, respectively for: normal heart, TR3ISVR™ procedures and Myosplint procedures

The modelling of Ventricular Restoration procedures was performed in two steps:
1) Structural Mechanical Dynamics, based on the FEM-method (ANSYS 11), used for studying the deformation of the myocardium and the papillary muscles.
2) Computer Fluid Dynamics with Fluid Structure Interaction (FSI two-way coupled methods) for studying the influence of the patch and splints on flow pattern, myocardial stress and ejection fraction of the elliptical LV model.

The shape, structure and function of the heart (especially with HF) are very complex. The present state of knowledge does not facilitate the exact imaging of the heart's function. As the comparison of the two procedures is impossible in clinical conditions, modelling methods were used applying the same physical conditions. For computational simplicity only some features of the heart's function were selected. Generally, the myocardium is a non-linear viscoelastic anisotropic active material [10]. Active tension is generated by cardiac muscle fibres. The deformation of the myocardium (heart muscle) is represented by the strain tensor. The complex movement of the heart wall can be seen as the superposition of torsion and both radial and longitudinal contraction. For computational simplicity, the isotropic linear elastic model of the myocardium was used where the stress and strain relationship followed Hooke's law. For all 3D computer models, the same diastolic volume (160 ml) and wall thickness were obtained with the following basic mechanical parameters of the myocardium: isotropic linear Young's Modulus 4 MPa, Poisson's Ratio 0,42.

The FEM Mesh contained max.120,000 nodal points. The influence of the patch on the flow pattern and myocardial deformation was examined for two values of elastic modulus of the patch: 100 MPa and 400 MPa.

RESULTS

In the TR3ISVR™ procedures, the mechanical properties of the ventricular patch influence heart wall dynamics. The stiffness of the Dacron patch (<300 MPa) is significantly different from the myocardial wall, which caused a change in the LV shape (especially in the end-systolic phase), disturbance of wall dynamics, significantly increased stress near the patch and decreased Ejection Fraction. Young`s modulus of the patch increased from 100 MPa to 300 MPa, the Ejection Fraction decreased by approx. 16% and the myocardial stress increased by more than 50% (Fig.6).

The application of Myosplint devices reduced the LV volume, but in the region of epicardial pads, the myocardial stress increased by over 100%. As demonstrated by computer simulation (FEM analysis), the use of the Law of Laplace causes incorrect description of LV stress redistribution after the application of the Myosplint system. Wall (myocardium) stresses increase significantly within the Myosplint pads. (Fig.4, Fig.6).

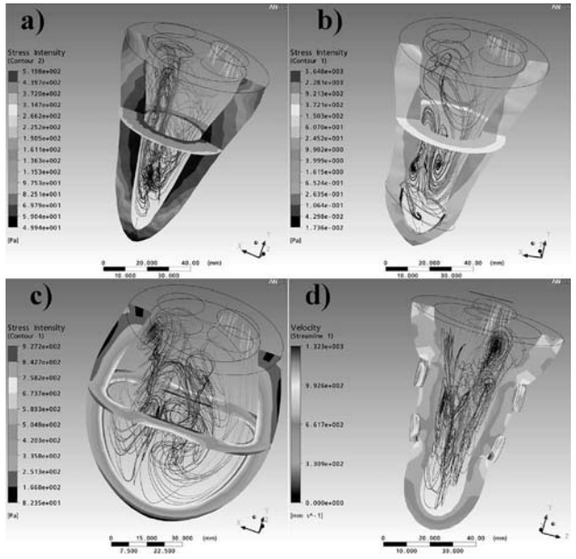

Fig.4. Myocardial stress and streamline of velocity in the end-systolic phase for:
a) normal heart,
b) heart afterTR3ISVR™ procedures,
c),d) heart after Myosplint procedures from FSI-simulation

The initial strain and elastic properties of the Myosplint cords also influence the LV wall movement. Too high initial tightening of the chords can cause significant LV deformation in the end-systolic phase and decreased EF. During the entire cardiac cycle, both in the diastolic and systolic phase, the myocardial tension was at maximum values (Fig.5).

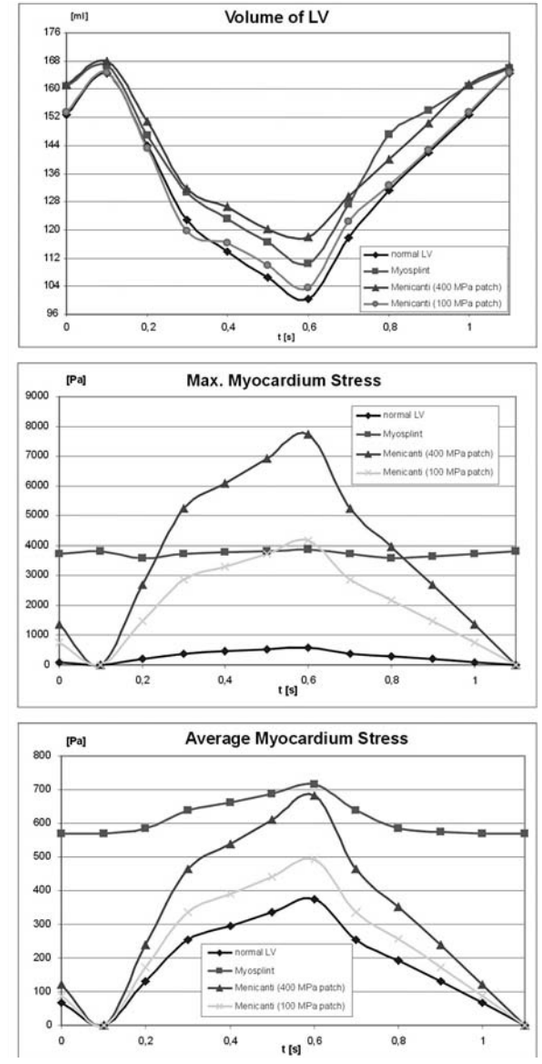

Fig.5.Volume, maximum and average LV myocardial stress for: Normal, Myosplint procedures and TR3ISVR™ procedures from FSI simulation

Additionally, due to the flattened shape of the LV and the use of Myosplint cords, contractions occur mainly in the short-axis direction, thus making LV twisting difficult.

Mitral valve regurgitation can be caused by the widening of the mitral annulus and the dimensional distance between papillary muscles resulting from LV deformation [13]. The very high patch stiffness in TR3ISVR™ procedures caused the distance between the papillary muscles to be enlarged (both at the base and the top of the muscles) during systole. In the LV with the Myosplint system, this distance is smaller (Fig.6).

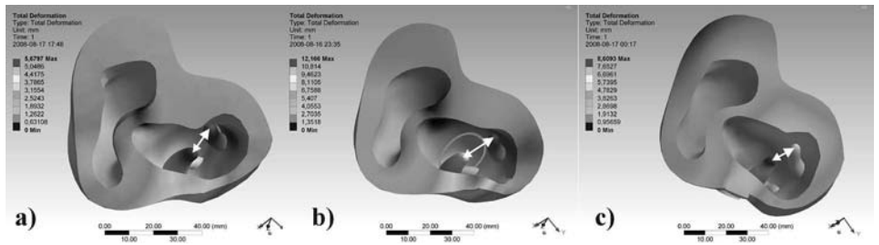

Fig.6. Deformation of myocardium and papillary muscles in the end-systolic phase for:
a) normal heart,
b) heart after TR3ISVR™ procedures,
c) heart after Myosplint™ procedures

Papillary distance [mm]	Normal	TR3ISVR™ procedures E_{patch}= 100 MPa	TR3ISVR™ procedures E_{patch}= 400 MPa	Myosplint™ procedures
peak	11,98	15,09	17,15	10.73
base	23,20	26,90	28,32	21,47

Table1. The distance between papillary muscles for normal heart and heart after TR3ISVR™ and Myosplint™ procedures

The use of a stiff patch (400 MPa) can increase the distance between the peak of the papillary muscles by over 50 %, in relation to normal heart ventricle (Table.1). It can significantly influence mitral insufficiency [13,14].

CONCLUSION

Both methods of LV reconstruction in patients with Congestive Heart Failure generally reduced the LV volume. The Dacron patch and the Myoslint pads can disturb LV wall dynamics by decreasing EF and increasing myocardial stress. The main disadvantage of the Menicanti procedure is the placing of synthetic tissue inside the LV cavity. A stiff Dacron patch can cause mitral regurgitation due to the widening of the distance between LV papillary muscles.

In order to improve heart wall dynamics, patches with small elasticity modulus (pericardial patches) are preferred. Myosplint procedures belong to minimally invasive surgery but reconstruction of the correct shape is impossible. Additionally, Myosplint pads increase myocardial stress and disturb LV wall movement. The selection of CHF patients for Left Ventricular Reconstruction is very important. Hence, computer modelling of LV reconstruction procedures will help to restore the shape, volume and wall dynamics of the damaged ventricle in a more precise manner, and thus improve the early and long term results in patients with Congestive Heart Failure.

REFERENCES

1. Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine, 8th ed. Copyright © 2007 Saunders, An Imprint of Elsevier.
2. William Dec G. Heart Failure. Marcel Dekker, 2005, New York, USA.
3. Lorenzo Menicanti, M.D. and Marisa Di Donato, M.D. The Dor procedure: What has changed after fifteen years of clinical practice?, The Journal Thoracic and Cardiovascular Surgery 2002;5;886-890.
4. Dor V, M, Sabatier M, Montiglio F, Civaia F. Di Donato Endoventricular Patch Reconstruction of Ischemic Failing Ventricle. A Single Center with 20 years Experience. Advantages of Magnetic Resonance Imaging Assessment. Heart Failure Reviews, 9,269-286, 2004.
5. Dor V. Left ventricular reconstruction: the aim and the reality after twenty years.
6. J Thorac and Cardiovasc Surg. 2004;128: 17-20.
7. Menicanti et al. Surgical Ventricular Reconstruction.Seminars in Thoracic and Cardiovascular Surgery, Vol. 13, No. 4, Oct. 2001.
8. R.Przybylski, M.Zembala, T.Kukulski. Nowa metoda chirurgicznej rekonstrukcji lewej komory serca sposobem Menicantiego. Kardiochirurgia i Torakochirurgia Polska 2004. 1: 64-70.
9. Xavier M. Mueller. Reshaping the remodeled left ventricle: a new concept European Journal of Cardio-thoracic Surgery 20 (2001) 786–791
10. Sahn D.J, Vick G.W. Review of new techniques in echocardiography and magnetic resonance imaging as applied to patients with congenital heart disease. Review Heart 2001;86; 41–53
11. Ed. Joseph D. Bronzino. The Biomedical Engineering Handbook: Second Edition. Boca Raton: CRC Press LLC, 2000
12. Regen DM. Calculation of left ventricular wall stress. Circ. Res. 1990;67; 245-252
13. Yin F.C. Ventricular wall stress. Circ. Res. 1981;49;829-842.

14. di Donato M, Sabatier M, Dor V, et al, Effects of the Dor procedure on left ventricular dimension and shape and geometric correlates of mitral regurgitation , J Thorac Cardiovasc Surg 2001;121 91-96
15. GE Burch & TD Giles, Angle of Traction of the Papillary Muscles in Normal and Dilated Hearts: A Theoretical Analysis of Its Importance in Mitral Valve Dynamics, American Heart Journal 1972; 84: 141-4.

The Ethics of Artificial Organs

Zbigniew Nawrat

I believe ethics to be the art of making the right choices.

And modern medicine, with its new medical techniques, healthcare systems and financing schemes, creates a completely new area of insecurity in this regard. For ages, philosophers have been analysing issues connected with the man-versus-man and man-versus-the world relations in order to help us comprehend the reality and find the correct conduct. Before moving onto the moral and ethical dilemmas connected with advances in medicine, let us look at the terminology and the biographical outline relevant to this field. The choice is subjective – essential in the search for inspiration.

The term "ethics" comes from the Greek word *ethicos,* meaning a way of conduct accepted in the society, a conduct according to the legal character (*ethos*-character). Today, "ethics" is colloquially understood as "morality", although the Latin word *moralis* denotes more the judgement of the appropriateness of a given action than a person's character.

Plato (427-347 BC), a known opponent of democracy, believed that most people live in ignorance, and therefore cannot be expected to make the right decision. Knowledge is a virtue, nobody purposefully chooses the wrong way.

In his Nicomachean Ethics, Aristotle (384-322 BC) identified two types of virtues: moral (courage, generosity, modesty, etc.) and intellectual (wisdom, intelligence, reason). We all have the possibility to develop a virtue, however, only few are successful.

Thomas of Aquin (1225-1274) perceived moral problems in the context of the law of nature and God's commandments. A moral life is life "in accordance with reason". All people are equal and for all people there is a close connection between happiness and a righteous conduct (conscience).

Immanuel Kant (1724-1804) believed that a man's kindness does not depend on the effects of his actions, since there are too many factors which influence these actions and which we cannot influence. The development of the good will is the most important human aim.

Let us mention just two imperatives all human actions are subject to:

1. "Act as if the maxim of thy action were to become by thy will a universal law of nature."
2. "Act in such a way that you treat humanity, whether in your own person or in the person of any other, never merely as a means to an end, but always at the same time as an end" (the so-called practical imperative).

Jeremy Bentham (1748-1832) formulated a concept of utilitarianism according to which, one should be useful and act in a way that would cause the greatest good for the greatest number of people. His principle of utility regards "good" as that which produces more pleasure and less pain. Act utilitarianism means that it is the results or consequences of a given act that decide on its moral value, and not the reasons or motivation behind that act.

A.J. Ayer (1910-1988) and CL Stevenson (1908-) created the theory of emotivism. According to this theory, the scientific, empirical method of verification of statements is inefficient in ethics, whilst morality is only connected with our feelings.

The contemporary British philosopher, Alasdair MacIntyre, in his search of understanding of good and evil delves into history and art, such as: the Homeric virtues (strength, courage, cleverness, friendship), Athens' virtues (courage, friendship, moderation, wisdom), and medieval virtues (bravery, justice, moderation, wisdom, faith, hope and love).

Joseph Fletcher considered moral issues in three ways:

- legalistic (based on unknown commands);
- autonomic (rejecting rigid norms);
- situational (e.g. "love thy neighbour").

His situational ethics suggests that decision making should be based on predicting the consequences of a given act. This, however, is not always possible.

John Harris, in his 1985 book "The value of life" claims that two issues should be taken into account:

1. the problem of "equal opportunities" (e.g. a child has a longer future ahead than an old person)
2. the problem of "quality of life" (e.g. once recovered, a younger person hasa more active life ahead).

Healthcare administrators use the Quality-Adjusted Life Year Schedule (QALYS) to assess the value for money of medical treatment. This method may be suitable for discussions with the insurer, but it does not solve ethical problems at all.

The Universal Declaration of Human Rights (1948) adopted by the United Nations Organisation recognises:

- the right to life, liberty and security of person,
- the right to a standard of living adequate for the health and well-being of himself and of his family, including food, clothing, housing and medical care and necessary social services.

But we still do not have the universally correct answers to these questions:

1. Should doctors resort to extraordinary measures to support a patient's life, when the life offered cannot be a dignified one?
2. Can doctors shorten a life that has become so burdensome and humiliating that the patient, fully aware of his actions, asks to put an end to his sufferings?

How to choose between two people in need in the face of insufficient means? And what will happen once we are finally able to live infinite lives? There is no point escaping from such questions: sooner or later, specific methods will have to be introduced in order to regulate the availability of life. By introducing a fair choice to die, we will be able to eliminate more effectively the earthly pain and suffering.

According to Erich Fromm, modern society is characterised by the individuals' tendency to free themselves from social limitations: from the authority of Church and the control of local communities. But at the same time, the individual begins to feel isolated and lonely.

So, where does a man's freedom begin? Is it where his loneliness begins?

However, most people choose to escape from freedom for fear of having to face their own creativity, individualism and … loneliness, or is it responsibility? Fromm described three common escape mechanisms: authoritarianism, conformity and destructiveness. With the first, an individual gains a sense of power, but loses his own independence and identity. A person choosing conformity, loses his ability of critical thinking.

Until not long ago, it was the family that guarded a man's social and psychological security. We are trying to create administrative and cultural substitutes that form the framework of today's "urban existence". But we sense the truth in Albert Einstein's statement that "the unleashed power of the atom has changed everything, save our modes of thinking". Parables from the Bible are still relevant. The civilisation of permanent technological development imposes life in city centres, with a decreasing role of family settlements. On the one hand, it separates multi-generational families through the physical distance between the parents' and their adult children's places of living, and yet on the other hand, it draws them closer thanks to modern means of communication.

The use of robotics in the treatment process facilitates remote medical care, consultations and the monitoring of a patient's condition. It is therefore a chance for a greater availability and quality of medical services. Robots are a breakthrough in the infrastructure, the organisation of the operating theatre and in the specialist training of surgeons. But how to evaluate a wrong decision or action of a doctor at a distance? How to divide the responsibility for the mistakes of remote robotic devices? The access to information depends on technical resources, software, etc. Therefore, the final effect is influenced by a number of people – engineers, administrators, economists, etc. as well as fortuitous events. We should not be misled by the fact that the amount of objective information concerning the patient's condition is now much greater and of much higher diagnostic value than a few years ago during a classic visit to the doctor. Ethics and morality mutely assume a human-to-human contact. Our conscience and empathy work differently in the absence of a direct connection between our actions and their effect on others. To what extent is the aviator that dropped the atomic bomb on Hiroshima guilty? Who should be superior in decision making – the computer or the man? Similar issues appeared several dozens of years ago in military politics and … aircraft controls. Generally speaking, today we have two equally good (or equally bad) systems: the last word belongs to the man (American) or to a computer-based advisory system (European). The technical resources facilitated an increasingly extensive impact on the environment, but at the same time multiplied the hazards.

Today, doctors receive the diagnostic results, frequently with an automatic expert's note, on the basis of which decisions are made. Tomorrow, this will become a supervised obligation, like doctors' decisions controlled by insurance companies. It is true that so far, medical decisions have been made by people but people who are incapacitated, or if you wish, supported by medical standards. Standardisation is usually based on reliable statistical research. So, has medical ethics become the result of statistical and economic research?

Our society is ageing. This changes completely the outlook on healthcare and the recommended directions of its development. As in the face of a crisis, we should encourage the development of methods and technologies that can be successfully disseminated among a large group of cheap and effective recipients. Luckily, the new technological solutions may decrease the costs of production and use, as well as improve the functional properties of many medical devices and applications. We are presently using far less material per each megabyte of processed information than several years ago. The International Futures Programme predicts the introduction of completely new devices into medicine in 2030 as a result of advances in nanotechnology, e.g. intelligent drug carriers, micropumps and nanorobots. The possible uses of nanotechnology are vast and are likely to play an important role in our future economic prosperity and quality of life. However, the risks are considerable, therefore new codes of conduct are being created with regard to responsibility in nanoscience and nanotechnology research.

The high index of ageing of the society, common heart and circulatory diseases, neoplastic diseases, locomotor system diseases as well as locomotor organ traumas resulting from motor vehicle accidents, all create the need for devices replacing and supporting the inefficient organs. The technological progress in biomedical engineering and regenerative medicine constitute the basis for innovative artificial organs; adequately small, efficient, durable and energetically and mechanically functional.

A new generation of Micro-Electro-Mechanical Systems (MEMS) is entering medicine. The fast development of bioelectromechanical microsystems (bioMEMS), micropumps and bioinformatics has created new possibilities, such as the "lab on a chip" micro-laboratory, which are revolutionising diagnostics and therapy. For example, a suspension of quantum dots (when induced, they emit light of precisely determined wavelength) with attached "addresses" (particles recognising tumour cells) introduced into the circulation, will in future facilitate the detection of neoplastic changes in the earliest, pre-symptomatic stages of development. In the nearest future, technical devices, entirely artificial organs and robots will be used to secure tissue therapy and genetic therapy. However, the diagnostic success of screening and specialist examinations means an increase in the absolute number of patients undergoing the treatment process, that is treatment at various stages of prevention of the expected effects of the developing disease and their elimination. How, in the face of finite and always insufficient financial means, will decisions be made

as to their optimal use? Today medicine deals mainly with the treatment of diseases revealed by incorrect physiological features. But it is much cheaper to prevent. And what about the responsibility of a man, who through incorrect conduct (diet, stimulants, etc.), exposes the society to the potential risk of having to cover the costs of his treatment? Where is the borderline between individual and collective freedom? Who, and according to what criteria, will make the decision that Mr X is to be kept alive at the cost of one million Euro a year, Mr Y will not receive first aid, whilst Mr Z will be living at a 63% risk of developing a lethal disease (the same as Mr X) at the age of 44?

What will be the results of relying more and more heavily on technical means with regard to the length and the quality of life? In the past, the quality of life was evaluated on the basis of access to potable water. Today, we add access to electric energy and information. In the times when philosophy and ethics were born, an individual was not as strongly connected with his social and technical environment. Today, we are a mesh in the global net. Thanks to advances in medicine, we have growing numbers of patients as a result of defective genetic codes being transmitted to the offspring. Diabetes is a typical example. We stop viral, infectious diseases. Today, African governments are heavily dependent on AIDS drugs. To what extent are our decisions ethically objective, in conditions of a strong dependence on so many elements of the "net"? So much depends on the distribution of means of subsistence. Simple objective simulation models suggest that present decisions on the level of home, country, company are critical for the existence of the civilisation.
Imperceptibly, a change has taken place; we are all consumers and less and less citizens. We are taking decisions concerning the future of the world by undertaking market actions, that is buying. We give our vote proportionally to our resources. But financial resources have little to do with moral or ethical principles. In our times, Jesus would be put in prison for evasion of taxes on healing and on feeding the hungry.

After Frankenstein and robots, cyborgs are yet another imaginary creation that is entering reality. I have recently met an eccentric English scientist, Kevin Warwick, who is the first man in the world to have autoimplanted into his forearm the so-called electronic chip enabling the transmission of information from and to the nervous system. By implanting a similar electronic system into his wife, he was able to communicate with her also telemedically. Undoubtedly, soon we will deal with implantation of specialist chips increasing knowledge, memory, the possibility to communicate as well as artificial organs enhancing the professional abilities of doctors, police officers, etc. This way, it will

also become possible to implant the code of conduct and the discipline to follow it. **An ethics chip implant**. Already today, artificial organs facilitate the achievement of good results in competitions, as successfully proven by Oscar Pistorius, a runner with two lower leg prostheses who aimed to participate in the latest Olympics. Soon, it may become fashionable to enhance our senses and physical possibilities through electromechanical and electronic devices and artificial organs. How is this going to influence the definition of beauty, truth and happiness?

At Reading University, Prof. Warwick's team is currently doing a research with the use of naturally cultivated neurons as brains controlling small mechanical systems. The integration of engineering and biology is a fact. This raises the question as to the borderline between a biological organism and a technical device. Where is the beginning of consciousness and intelligence? In my opinion, it is when the question "why" appears between the information from the environment and the actions.

Until now, all choices have been made via biological evolution or civilisation-based solutions that regulated cohabitation and the division of goods of the citizens of a given area. The first revolution was the introduction of money. The separation of value from the object has contributed to a rapid acceleration of the development of mankind. Fast legs, so important in hunting, and the fire, previously vital for survival, have given way to the brain and accumulation of capital, including knowledge. Thanks to bioengineering and medicine, today we can influence the survival of certain individuals and entire groups of patients. Thanks to democracy, all people have an impact on the decisions taken. How will we use the chance to influence our own fate, that of our neighbours and that of our planet? By introducing artificial human components, do we just treat difficult cases or do we introduce completely new laws of nature?

Ethics is like traffic lights – it facilitates the distinction between right and wrong. In many professions involving considerable levels of responsibility, the accepted standards and laws constitute a protective shield for the employee and his actions. In a practical way, they enable the taking of a decision and its evaluation.

What can we learn from the teachings of philosophers? After all, despite the quickly developing knowledge, we are frequently ignorant (Plato) in the face of a disease and the awareness of the fact does not help us at all. Indeed, the doctor is by definition doomed to taking life-and-death decisions, despite

the usually complete information as to the patient's condition; however, from a philosophical point of view, we will never have all the elements of this knowledge. We develop intellectual and moral virtues although we are not always proud (Aristotle). Hence the value we put on awards, from sanctity to the Nobel prize. "In accordance with reason", we take decisions concerning our life and that of others, which have the same value (Thomas of Aquin). We are not able to influence everything, but judging by the results we can evaluate the effectiveness of actions (this is the essence of statistical research, even if we do not entirely understand the laws of nature). Kant's practical imperative is frequently quoted when faced with the need to find prudent solutions to problems, e.g. concerning the use of stem cells. Looking at our personal participation in the life of a community in any country, we believe in the sense of utilitarianism (Bentham); faced with the necessity to choose "the lesser evil", we will follow the principles of "equal chances" and "quality of life" (Harris). Whenever our actions may have a global influence on the environment or the mankind, we should always look through the perspective of Kant's imperative, as if we were to create a new law of nature. We, the humankind, are now too strong and too weak at the same time to make mistakes.

Artificial organs are not the most expensive part of medicine; they are its indispensable part, today and tomorrow. The use of intelligent artificial and biological materials and devices is actually cheaper than other means of target therapy (e.g. pharmaceutics). The key to success and full dissemination, however, is the minimal invasiveness of implantation and the automation of servicing and supervision. With the harmonious development of technical and biological sciences and their reasonable implementation, we can influence the evolution of species and the quality of life on Earth.

Throughout the world, there are doctors and a huge group of patients waiting for new technologies of protection against the consequences of diseases, for the chance of saving life and its good quality.

We are facing a century of completely new challenges. By introducing possibilities of saving life, we make real the hopes of new groups of patients. We must work on a system of gradual lowering of costs, since no social system can withstand the tension born between great hopes and the lack of possibility for their fulfilment.

A few years ago I wrote: "Every day, new branches of our common tree are created and even if not all of them bring fruit, the overall balance is positive.

We live longer, better, more happily. **Because in order to live, we need access to three resources: energy** (processing of matter)**, information** (intellectual goods, from religion, through science and culture to education) **and freedom** (social goods, such as our relationships with other people)**.** It is thanks to them, that we can feel safe, that we can develop and express feelings. New technologies and innovations should provide an easier access and utilization of these resources." Because they have been created to multiply our freedom.