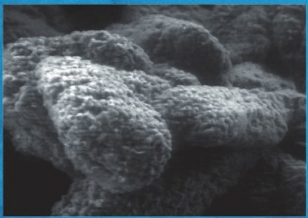
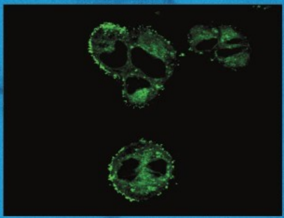
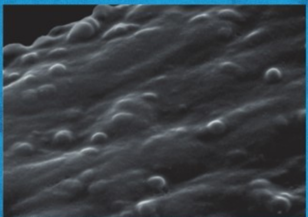
UPDATES in SURGERY

Alfonso Barbarisi

In collaboration with P. Bechi
P. Innocenti • C.A. Redi • F. Rosso

Biotechnology in Surgery


Springer

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Foreword by
Enrico De Antoni

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The publication and the distribution of this volume have been supported by the Italian Society of Surgery

ISBN 978-88-470-1657-6 e-ISBN 978-88-470-1658-3

DOI 10.1007/978-88-470-1658-3

Springer Milan Dordrecht Heidelberg London New York

Library of Congress Control Number: 2010935728

Cover design: Simona Colombo, Milan, Italy
Typesetting: Graphostudio, Milan, Italy
Printing and binding: Arti Grafiche Nidasio, Assago (MI), Italy

Printed in Italy

Springer-Verlag Italia S.r.l. – Via Decembrio 28 – I-20137 Milan
Springer is a part of Springer Science+Business Media (www.springer.com)

To my patients,
To Alberto, Anna, Titty,
To Caterina,
To Alberto, Manlio, Anna,
with gratitude and love.

Foreword

The Italian Society of Surgery has always had a great interest in biotechnological developments which provide new and appropriate solutions to current surgical problems.

In the last decade, at the annual Congresses, the Society has always aimed at treating biotechnological subjects in the knowledge that it will bring improvements to surgery in the 21st century, as was the case with technological development in the last century.

It is with great pleasure and interest that the Italian Society of Surgery offers its members and the whole medical community the opportunity to approach and to expand this branch of human knowledge for new applications in health care.

We therefore appreciate the efforts of our member Alfonso Barbarisi, President of the European Surgery Society (ESS) and Head of the Surgical Department of the Medical School at the Second University of Naples. Prof. Barbarisi has carried out research both in Italy and abroad for a long time with persistance, belief and philosophic attitude. The purpose of this work is to offer surgeons an approach to biotechnological techniques for their better use in every day surgical activity. The other goal is to attract biotechnologists to the complex query for *help* by medicine in the attempt to solve mankind's health needs in the best way possible.

I am convinced this book has a great scientific and practical value. It will make surgeons more conscious both in applying biotechnologies already in use and in more rapidly taking on future biotechnological discoveries in their culture. I am sure it will be accepted with enthusiasm by the Italian and international surgical community.

Rome, October 2010

Enrico De Antoni
President, Italian Society of Surgery

Preface

The 20th century has drawn to a close, the century when surgery took huge steps forward thanks to the progress of technologies. Now we are approaching the century of biotechnologies which will cause not only a progress in surgery, but a real *cultural revolution* which will completely change the approach to solving different problems in medicine. The aim of this book is to bring surgeons nearer to biotechnologies and to overcome the cultural gap dividing them.

A lot of biotechnologies have already been put forward and are used as a routine in surgical practice, to the complete unawareness of surgeons. It is important that surgeons realize this and understand the real mechanism of biotechnologies and their practical applicability, so they can be more aware of them and master their use.

A surgeon has always had a blinkered view of patient treatment because he has always suffered from the limits of his instruments. He has always been a technologist, in the sense that since the beginning of surgery he has always needed technology, from the scalpel to advanced surgical instruments. As a consequence he has always been a protagonist of technological progress, he has invented new surgical instruments, he has always modified them, he has used physical means (*iron* and *fire*); he has always cooperated with technologists.

In this new century, the first of the new millennium, an increasing amount of knowledge which is unusual to the surgeon's traditional technological training is encroaching upon surgery, hence the aim of this book. Now it is urgent to bring surgeons towards this knowledge (biotechnology) which by its nature is completely different from the technologies used so far, because it goes beyond the senses of sight and touch, which have been till now the essence of the surgeon's action.

The cellular and molecular dimension of biotechnologies is really by far the most advanced and modern surgical action. The use of bio-materials produced biotechnologically, the use of engineering tissues, the use of biomarkers is a routine that is not near the traditional action of surgeons who have a sort of distrust which lies not in a refusal of the new, but in poor knowledge of biotechnologies.

A solid, workable and cultural alliance between biotechnologies and surgeons is required, as has already happened in the past between surgeons and technologies.

A common language between surgeons and biotechnologists will bring further, revolutionary progress to surgical sciences in the 21st century, which is purported to be the century of biotechnologies.

Naples, October 2010 **Alfonso Barbarisi**

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The Philosophy of Biotechnology

1

A. Barbarisi

1.1 Introduction

Biotechnologies are those technologies living organisms use to keep themselves alive and which, once discovered, human beings use to make products useful to them. Biotechnologies are at the base of the phenomenon of *life* and so they are naturally present in nature. They are not invented by man, they are instead discovered while studying the phenomenon of *life*. So biotechnologies differ from technologies. Technologies are the result of man's intellect and creativity, which are used to invent machines and devices not present in nature, but produced to satisfy man's life needs.

The use of biotechnologies takes place using living organisms or parts of them to make large quantities of products useful to man, and this is one of the areas of high technology in great expansion and from which very interesting results for productive activities have come and are expected.

Many people think biotechnologies are recent developments, but in fact they have always existed and they have been used for thousands of years. Today, however, the huge progress in the knowledge of vital mechanisms at the cellular, sub-cellular and molecular level has made it possible to recognize and discover them in their minimal mechanisms and to take advantage of this knowledge widely and with great flexibility.

Biotechnologies have been traditionally used in productive activities, in agriculture, in zootechnics and in food production in general for a long time. In prehistoric times man prepared drinks and fermented food: the Sumerians and the Babylonians produced wine and beer as early as 6000 B.C. and the Egyptians produced leavened

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bread as early as 4000 B.C. In reality these forefathers of biotechnology were completely unaware of the processes taking place during the achievement of these products and they did not realize that the action of living organisms was used in them. It was only thanks to Leeuvenhoek's microscope that researchers managed to observe the microorganisms responsible for the production of beer and leavened bread. And only Pasteur, between 1857 and 1876, understood the general mechanisms of beer production and identified the mechanisms making it possible.

After the discovery of a new substance produced by microorganisms – penicillin – Fleming laid a milestone with the discovery of a new class of drugs: antibiotics. However, the ability to produce antibiotics in large quantities needed another biotechnological passage: the selection of a microbial species able to produce these substances in greater quantities than the species found in nature.

In modern times, the development of biotechnologies has become extremely rapid. Their fast and widespread use has caused, both in their applications and in the same fundamental research, considerable and inevitable ethical problems regarding the protection of biodiversity and the validation of the biotechnological product.

One of the pivotal points of the new philosophy of the current knowledge on biotechnology is to let clinicians, surgeons and all its most recent users approach and face the problems and the requests of today's medicine and surgery. The main question is not to know the numerous biotechnological discoveries, but rather to understand their original application to the problems of medicine and surgery. This approach tries to show application solutions to the use of biotechnologies.

At present new technologies producing new vaccines or improving existing ones are available. Monoclonal antibodies are another biotechnology achievement which have found particular application in prevention, diagnoses and therapy thanks to their pureness, specificity and unlimited disposal. Gene therapy is another biotechnology application. This term refers to the transfer of genetic material to prevent or treat a disease. In the case of genetic diseases, for example, where a gene is dysfunctional or absent, this therapy allows the transfer of the *right* gene to the sick organism, so as to correct the defect. By using the same gene manipulating techniques used in plants, we have discovered we can intervene even on the DNA of more complex organisms, from bacteria to superior mammals, with the creation of transgenic animals. These animals are used in different areas: in the biomedical field, for example, they produce man's life-saving drugs in their milk, or they form the basis of experimental models which are indispensable in acquiring knowledge of very severe diseases. In the medical-surgical field investors are also concentrating their attention on the realization of transgenic animals for xenon-transplantations.

Another very current biotechnological application is in the field of stem cells. Man's body tissues are composed of different cells, each one with its own specific characteristics. However all of them derive from the primordial cell coming from the fusion of the male and female gametes during fertilization. At the very beginning of embryo development, each cell is totipotent because it can give rise to any of the 254 different cellular types present in an adult organism.

Tissue engineering is an emergent interdisciplinary research field which applies the methodologies and the techniques typical of material engineering (technologies) and life sciences (biotechnologies) to understand and solve the problems linked to tissue repair and substitution in living organisms (prosthetic-substitution surgery).

The major improvement in the knowledge of the structure-function relations in biotechnology and in medicine and the recent developments obtained in chemical, material and bioengineering have in fact made possible the design of substitute functional tissues. This approach uses living cells associated with biomaterials in which, or on which, cells can proliferate, organize, and differentiate themselves in a manner similar to native tissue and thus three-dimensional organized tissues similar to the natural element to be rebuilt can be obtained. A new frontier in this field is the development of transplantable organs.

However, I think that in addition to the improvement of biotechnological knowledge, an appropriate philosophy of this knowledge is the strong trend in the use of biotechnology, rather than simply using technology for the solution of health problems.

Man has always tried to produce technologies to *subdue* nature and to defend himself from its adverse manifestations. This has given life to a competition which apart from being a failure has created a *vulnus* in the natural organization of things both in general and in the human organism.

The use of phenomena and of natural vital mechanisms to *correct* nature's deviances, that is to say diseases, is the great conceptual revolution of the *biotechnological way* to health. We could say that this way is an ecological approach to disease.

Various examples can be reported in surgery. Hemostasis can be obtained through electro-coagulation or other physical, very advanced and efficient methods, or through high local concentrations of coagulation factors, concentrations not present in nature, which highly accelerate the natural coagulation phenomena and produce immediate hemostasis. Through tissue engineering we can have completely or largely *natural* prosthetic substitutions, versus inert or not perfectly integrated prosthesis.

This is the philosophy of the biotechnological approach in medicine and surgery: to consider its great value in leading and letting us be led by nature itself, by vital phenomena in order to correct its own deviances, which in medicine are diseases and their negative consequences.

In the past century we have spoken about a pathophysiologic approach to surgery, where we have tried to use surgery to correct disease without producing major resection and destruction of soma. This philosophic statement was the longing of the surgeon who was nearer nature and less proud of the deep and physical upset he produced, and entitled to do so only by the higher interest in saving life.

To know the phenomenon of *life* in its deepest mechanisms and to find in it the help needed to change deviated nature into the natural and harmonic nature of wellness is the fundamental point of the union between surgeon and biotechnologist. The way we have traced cannot yet be taken in its widest development and moreover it is more backward than the technologic way, especially in surgery.

Surgery has become an exercise requiring very high technologic expertise. The situation of the surgeon today is similar to that of a fighter pilot who has gone from being a bold commander of a jolt vehicle to becoming an aeronautical engineer surrounded by a huge array of equipment in the air cockpit who to fly his craft has to control the different monitors and not simply look through the windows.

The surgeon, who has always been a technologist because he has always used instruments, even simple ones such as the scalpel, and physical actions such as the fire, has now become the user of numerous technologies present in the operating room and for whom even viewing the surgical field with his own eyes can be limiting, compared with the view offered by systems of navigation and magnification.

Now the biotechnology revolution has entered the operating room and the surgeon has to know, recognize and master it because he is at the summit of his mission: to cure the patient lying on the operating table. Or better still he has to be the fusion point to accomplish his mission. Once again he has to evolve, transform and surpass himself.

The first step in doing so is to believe in this philosophy, to learn the language of biotechnology and to speak with biotechnology researchers. Only after a confrontation and after understanding each other will they be able to use, rapidly and exhaustively the biotechnological discoveries which may risk going unused and losing the objective of the research: human welfare.

This is why the surgeon needs to know biotechnologies, those he already uses in his hospital department every day and those from which he can receive a great help in the future. Only in this way the benefits of biotechnologies will reach the patient early and in the best way. To do this can be expensive or more expensive than a traditional technological approach, but the surgeon must be convinced that biotechnologies and the integration between biotechnology and technology are the way ahead for the future and that they have the added value which at present justifies all financial efforts.

1.1.1 Genomics (fundamentals)

Genomics is the branch of molecular biology which deals with the study of the genome. In particular, it deals with the structure, sequence, content, function and evolution of the genome.

The term genome refers to all the genetic material (DNA or RNA) of an organism needed for survival and replication. Genomics was born in the 1980s when Fred Sanger sequenced the entire genome of a virus and a mitochondrion. In 1986 a public sequencing project began under the name of Human Genome Project with the aim of sequencing the entire human genome, but it was beaten by Celera Genomics (private company) which presented its results a year earlier (2000) than the public project.

The goals of genomics therefore includes the establishment of comprehensive genetic and physical maps of the DNA of living organisms with its complete sequencing.

A very important branch of genomics is transcriptomics, which deals with the expression of genes (mRNA) of a whole organism or a particular organ, tissue or cell at a particular point in the development of the organism or under particular environmental conditions, making major use of microarrays.

A DNA microarray, also called gene chip, DNA chip, or biochip, consists of a collection of microscopic DNA probes attached to a solid surface like glass, plastic, or silicon chip forming an array. These are used to examine the expression profile of a gene or to identify the presence of a gene or a short sequence in a mixture of many genes.

The segment of DNA linked to a solid support is known as a probe and thousands of probes are used simultaneously in an array. This technology arose out of a simpler technique known as Southern blotting, where fragments of DNA attached to a substrate are tested by gene probes with known sequences. The measurement of gene expression using microarrays has substantial interest in the field of basic research and in medical diagnostics, especially for genetic diseases where the genetic expression of normal cells is compared with cells affected by the disease in examination.

Recent developments in gene cloning and sequencing have made possible the identification of genetic causes of many diseases caused by mutations such as triplets (Friedreich's ataxia, X-fragile, myotonic dystrophy) and punctiform (sickle cell, Apert's syndrome) amplification.

1.1.2 Proteomics (fundamentals)

Proteomics is the science which studies the cellular proteome. The proteome is the set of all proteins expressed in a cell, including all isoforms and any possible post-translational modifications. The proteome changes over time, varies in response to external factors and is different between different cell types of the same organism.

Proteomics can be divided into three strands of research:

- systematic proteomics: identifies and characterizes all proteins;
- differential proteomics: studies the differential expression of proteins in different cells of the same organism and in different moments in the life of a single cell;
- functional proteomics: includes the study of interactions between proteins (interactomics), the study of interactions between a protein and its substrates (metabolomics) and the study of specific functions of proteins.

Proteomics is more complex than genomics because the genetic makeup is always constant in all cells of the same body and while about 30,000 genes in the human genome have been identified there are more than 100,000 proteins in human cells.

The study of proteins is performed mainly through biochemical assays such as: Western blot, electrophoresis (2D, SDS-PAGE), ELISA assay, immunoaffinity chromatography, microarray etc.

There is a large number of practical applications of proteomics: pharmaceutical, basic research, identification of new tumor markers, identification of a protein involved in a disease, study of its conformation and function to develop a specific drug (i.e. inhibitor) etc.