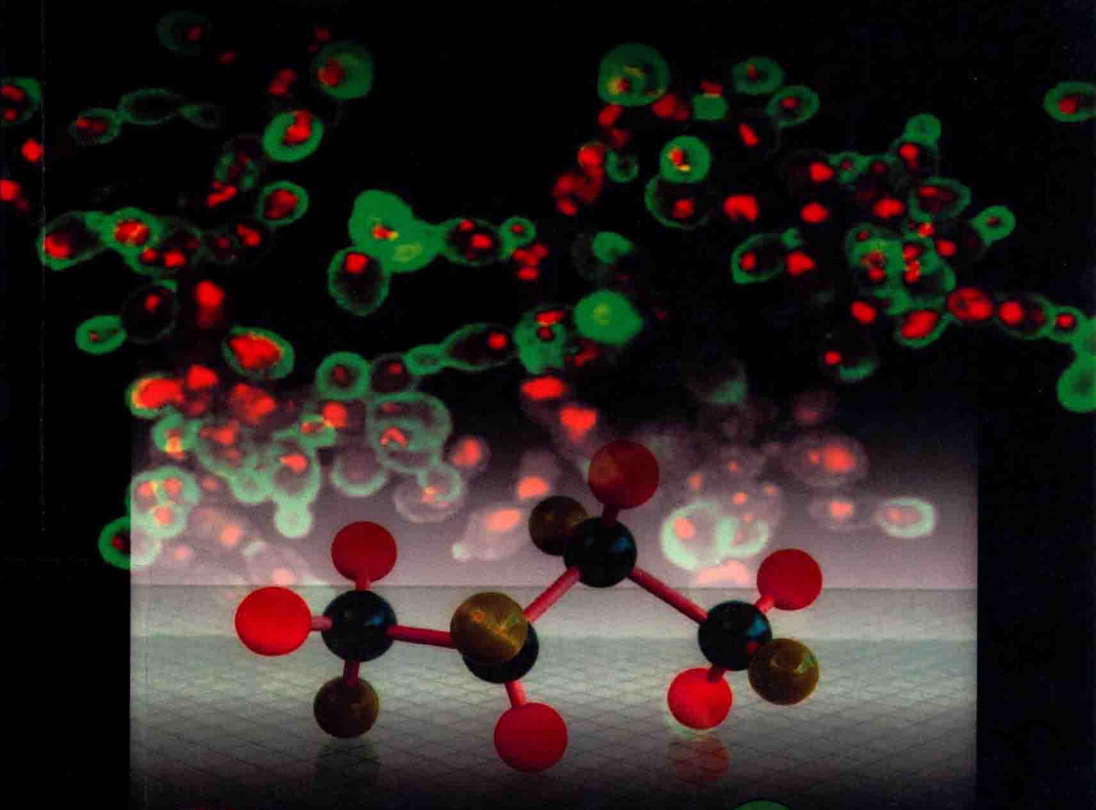
Second Edition

Cell Engineering

Li Zhiyong



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Preface

Cell engineering is a cutting-edge biotechnology that covers a very wide range in modern biological engineering and plays an increasingly important role in biology, agriculture, medicine & pharmacy, food science, aquaculture, environmental protection etc. Therefore, cell engineering forms an important and,part of the core curriculum for bioengineering, biotechnology and other majors at the college and university levels.

The first edition of *Cell Engineering*, published by the Science Press, China, in 2003, has been selected by a number of colleges and universities as a textbook or a reference book for teaching undergraduates or for graduates who prepare for postgraduate entrance examination or reexamination. The second edition was selected as a national teaching material for Chinese higher education in the 11th Five-Year Plan. The second edition of the book, still with the cell engineering technology as the main thread, comprises three parts: fundamentals of cell engineering, plant cell engineering, and animal cell engineering. While importance has been attached to comprehensiveness and systematicness, every effort has been made to render the language and contents as succinct as possible. In addition, an emphasis has also been put on extracurricular extended learning, so each chapter is followed by materials for self-learning by students and also for selective use by teachers in class. We welcome all suggestions and criticism from teachers, experts and students, for improvement of the book during its subsequent edition.

Li Zhiyong
Shanghai Jiao Tong University
December, 2012

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Chapter 1

Introduction to Cell Engineering

1.1 Bioengineering

Bioengineering or biotechnological engineering (including biological systems engineering) is the application of concepts and methods of biology (and secondarily of physics, chemistry, mathematics, and computer science) to solve real-world problems related to the life sciences and/or the application thereof, using engineering's own analytical and synthetic methodologies and also its traditional sensitivity to the cost and practicality of the solution(s) arrived at. Bioengineering is a comprehensive technology based on life sciences and applies biological systems and engineering principles to produce biological products and create new biological species.

1.1.1 Development of Bioengineering

1.1.1.1 The First Generation of Bioengineering

Bioengineering can be traced back to winemaking techniques 4,000 years ago. In 1674, bacteria were observed by Antonie van Leeuwenhoek with a microscope invented by himself; and in 1857, it was confirmed by Louis Pasteur that alcoholic fermentation was caused by yeast, revealing the nature of fermentation.

The period from the end of the 19th century to the 1930s witnessed the advent of lactic acid, alcohol, bread yeast, acetone, citric acid, amylase and other fermentation products, signifying the birth of the most genuine bioengineering represented by industrial microbial fermentation products. Most products in this stage came from the anaerobic fermentation process and remained with primary metabolites, thus the production process was relatively simple, not demanding on the equipment and small in scale.

1.1.1.2 The Second Generation (Modern) of Bioengineering

Modern bioengineering was formed during Word War Ⅱ in the 1940s, when drugs effective in resistance against bacterial infection with fewer side-effects were called for. Although penicillin was discovered by Fleming (British) in 1928, and manual extraction was realized and its healing efficacy clinically was proven by Howard Florey, Earnest Chain, and others in 1941, yet its large-scale preparation had not been achieved. In 1941, the U.S.A and the U.K. initiated a cooperation project on scaled-up penicillin production technologies, and then, the large-scale process of preparation of penicillin by fermentation was established. Streptomycin, chlortetracycline, neomycin and other substances soon came out one after another. The rise of antibiotic industry signified a new stage in the production of industrial microorganisms.

The experience in the production of antibiotics soon promoted the development of the amino acid fermentation industry in the 1950s as well as the enzyme preparation industry in the 1960s. Compared with the first generation, bioengineering products in this period were characterized by:

(1) Diversified product types, including primary and secondary metabolites, biotransformation/enzyme reaction products, etc.

(2) Exact demands on technology, significantly improved vitality and performance of strains; production with pure breed or under sterile condition; and aerobic fermentation in most processes.

(3) Huge scale, with some of the fermentation tanks weighing several tons.

1.1.1.3 The Third Generation (Contemporary) Bioengineering

In 1953, the double helixes structure of DNA was discovered by Watson and Crick, and in 1974, gene transfer was realized for the first time by Boyer and Cohen. With the rapid development of such technologies as gene recombination, cell and tissue culture, enzyme immobilization, large-scale culture of animal and plant cells, modern bio-reactors, bio-pharmaceuticals and other products in the 1970s, bioengineering has entered a new stage of development—contemporary bio-engineering.

With genetic engineering, it becomes possible for people design new life entities in a laboratory, and in the meanwhile, foreign genes can be connected to vector DNA *in vitro* before being introduced into host cells to obtain the target products. DNA recombinant technology products being developed or in production include interferon, insulin, growth hormone, lymphokine, blood fibrinolytic agent, vaccine, thymosin, albumin, erythropoietin, thrombopoietin,

calcitonin, chorionic gonadotropin, anti-hemophilia factor Ⅷ, hepatitis B vaccine, etc. Meanwhile, contemporary bioengineering also has extensive application in medicine & pharmacy, food, chemistry, agriculture, environmental protection, etc., bringing about a new technological revolution to these industries.

1.1.2 Contemporary Bioengineering

Biology, chemistry and engineering, the three most fundamental disciplines in bioengineering (Fig.1.1), provide bioengineering with theoretical support, while bioengineering, in turn, can promote their development.

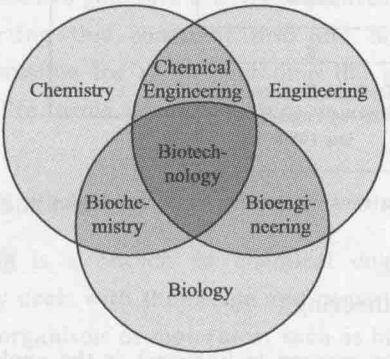

Fig.1.1 Relationship between bioengineering and other disciplines (Yu *et al.*,1991)

Traditional bioengineering included fermentation engineering, enzyme enginering, cell engineering, and genetic engineering, and later biochemical engineering, protein engineering, metabolic engineering and tissue engineering as well as other bioengineering technologies have seen rapid development. See Fig.1.2 for the development of contemporary bioengineering technologies and their relationship.

1.1.2.1 Fermentation Engineering

Fermentation engineering, also known as microbial engineering, refers to a technology that, with contemporary engineering techniques, exploits certain features of microorganisms to produce useful products for human beings or directly applies microorganisms to industrial production.

It is the oldest and was once the primary technology in bioengineering. Besides natural microorganisms, its objects also include genetic engineering microbes.

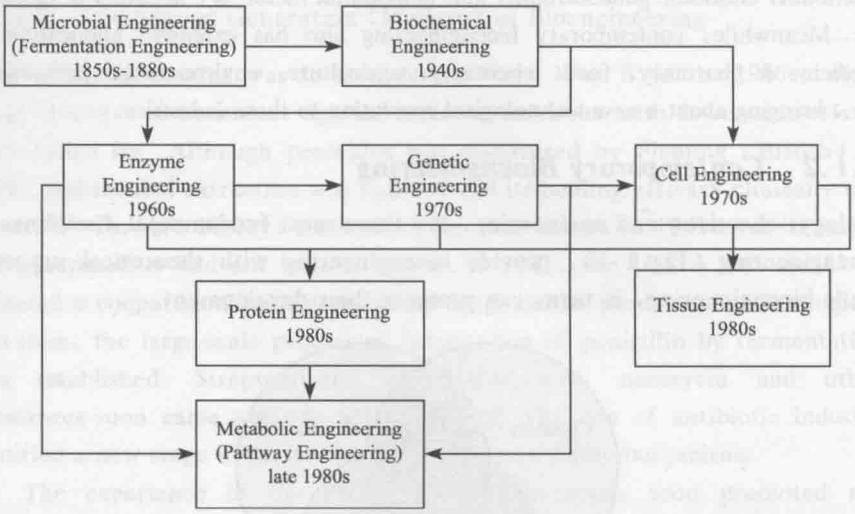

Fig.1.2 Relationship among six technologies in bioengineering

1.1.2.2 Enzyme Engineering

Enzyme engineering (or enzyme technology) is the application of modifying an enzyme's structure (and thus its function) or modifying the catalytic activity of isolated enzymes to produce new metabolites, to allow new (catalyzed) pathways for reactions to occur, or to convert some certain compounds into others (biotransformation). These products will be useful as chemicals, pharmaceuticals, fuel, food or agricultural additives.

Enzyme is a protein with catalytic activity produced by organisms, mainly including oxidoreductases, transferases, hydrolases, lyases, ligases and isomerases. Each can catalyze some specific chemical reaction, featuring high efficiency, mild conditions, reduced pollution and energy consumption, and easy control over the reaction.

1.1.2.3 Protein Engineering

Protein engineering is the process of developing useful or valuable proteins, that, based on genetic engineering, combines the basic knowledge of such disciplines as protein crystallography, computer-aided design, and protein chemistry to achieve manual targeted modification of genes and then modifies, reconstructs or splices proteins, so as to produce new proteins that can meet human needs.

Major research directions include: ①proteins modification at the genetic

level in view of creating proteins in new structure and with new functions, ②protein modification, namely genetic modification after protein synthesis. Since it is developed on the basis of genetic engineering, protein engineering is also known as "the second generation of genetic engineering".

1.1.2.4 Genetic Engineering

Genetic engineering also called genetic modification, is the direct manipulation of an organism's genome using biotechnology. New DNA may be inserted in the host genome by first isolating and copying the genetic material of interest using molecular cloning methods to generate a DNA sequence, or by synthesizing the DNA, and then inserting this construct into the host organism. Genetic engineering makes it possible for us to overcome the genetic barriers among species, to create new life forms absent in nature, and to meet the needs of the human society.

1.1.2.5 Biochemical Engineering

Biochemical engineering is a branch of chemical engineering or biological engineering that mainly deals with the design and construction of unit processes that involve biological organisms or molecules, such as bioreactors. Biochemical engineering is mainly concerned with general engineering problems in the conversion of laboratory research results into productive forces.

Biochemical engineering consists chiefly of: ①new bioreactor systems and production technology, ② development of new separation technologies and equipments, ③ description of the establishment of mathematical models of biological responses, and ④ production process online detection and control technologies.

1.2 Cell Engineering

Cell engineering is a comprehensive technology that applies the theory of life sciences together with the principles and technologies of engineering to purposefully make use of or modify genetic traits, so as to obtain specific cells, tissue products or new species.

Cell engineering is concerned not only with cells, but also with chromosomes, nuclei, protoplasts, fertilized ova, embryos, tissues or organs. According to biological categories, it mainly comprises plant, animal, and microorganism cell engineering. The latter is essentially fermentation or microbial engineering; therefore, it will not be discussed in the present book.

1.2.1 History of Cell Engineering

1.2.1.1 Exploration

Cell engineering dates back to the 19th century. Multinucleate tumor cells were discovered in vertebrates by Mahler in 1838. Roux, in 1885, found that nerve cells of chicken can survive in physiological saline and mentioned "tissue culture". In 1892, Driesch succeeded in isolating 2-cell embryos of urchins into single cells and collected complete larvae through tissue culture, and in the same year, the viability of embryonic single cells of lancelets was proved by Wilson. In 1907, American biologist Harrison isolated nerve tissues from the notochord of a tadpole and worked on culturing them in the solidified lymph of frogs, in which the nerve tissues survived for a few weeks, creating a precedent for animal tissue culture.

In 1902, German botanist Haberlandt proposed the theory of cellular totipotency and tempted to culture a single plant cell *in vitro*. In 1904, Hanig made experiments on embryo culture *in vitro* of radish and scurvy grass in inorganic salt and sucrose solutions. A preliminary success in pea, corn and cotton root and stem tip culture was made by Kotte and Robbins in 1922. And in 1937, the promotional effects of B vitamin and auxin on the growth of roots were discovered by Dutch botanist Went. From 1937 to 1938, French scientists Gautheret and Nobercourt almost simultaneously succeeded in culture of carrot tissues and proliferation of cells. Therefore, Went, Gautheret, and Nobercourt are claimed as the founders of plant tissue culture.

1.2.1.2 Inception

From 1956 to 1959, Swarup worked on low-temperature treatment of three-spine sticklebacks and finally triplonts were collected and fed until sexual maturity. The first animal fertilized *in vitro*-test-tube rabbit was collected by Chinese-American scientist Min Chueh Chang in 1959. Capstick and others succeeded in the suspension culture of hamster kidney cells in 1962, laying a foundation for large-scale animal cell culture technologies. In 1958, Japanese scholar Okada found that UV-inactivated Sendai virus could cause the fusion of Ehrlich ascites tumor cells, and in 1965, Harris and Watkins further proved that the fusion of animal cells could be induced by inactivated virus under appropriate conditions.

In 1948, Skoog found that adenine could induce the formation of buds, which he thought was determined to a large extent by the ratio of adenine to auxin. And in 1956, Miller collected a kinetin with higher activity than adenine

from isolating fish sperms and together with Skoog, proposed the formation of organs were controlled by phytohormone, and the ratio of auxin to cytokinin was the key factor that would determine plant cell differentiation: a higher ratio would be beneficial for the growth of roots, a lower one was conducive to differentiation of buds or stems, and a middle one would maintain division without differentiation. This discovery greatly promoted the development of plant tissue culture technologies. The possibility for somatic cells of carrots to differentiate into somatic embryos was discovered by Steward and Reinert in 1958, which was a major breakthrough in plant tissue culture and further validated the cell totipotency theory. The year 1960 witnessed the success in asexual propagation of orchids and other plants, opening up an effective way for rapid propagation of plants with tissue culture. In 1960, with fungal cellulase hydrolysis, Cocking successfully prepared plenty of protoplasts of cells from tomato roots and tobacco leaves, guaranteeing raw materials for the fusion of plant cells.

The renaming of *General Biology* as *Cell Biology* by Derobetis in 1965 marked the birth of cell engineering as a discipline, providing theoretical basis for cell engineering, and the continued improvement of animal and plant tissue culture, cell fusion technology, coupled with attempts on nucleus transfer, animal cloning, triplont breeding, *in vitro* fertilization, etc., ultimately contributed to the formation of this emerging discipline—cell engineering, around the 1970s.

1.2.1.3 Rapid Development

With the deepening of research in cell, developmental and molecular biology, biochemistry, genetics and other disciplines since the 1970s, cell engineering entered a rapid development stage. For example, the fact that polyethylene glycol can cause the fusion of plant protoplasts was discovered by Chinese Canadian Kuo-nan Kao in the early 1970s, signifying the preliminary establishment of the plant cell fusion technology. In 1981, Zimmerman applied variable electric field, a physical method, to induce protoplast fusion, further improving such technologies. Some significant achievements in this phase are as follows:

As for plants, $NaNO_3$ was applied as revulsant to induce the fusion of tobacco protoplasts and thus the first somatic hybrid plant in the world was breeded by U.S. scientist Carlson and others in 1972; in 1973, tobacco plants were cultivated by Nitsh; ginsenosides were produced by Furuya and others through cell culture in the same year, creating new ways for producing active substances of plants; the discovery of plasmid Ti contributed greatly to the

research on transgenic plants, bringing forth plenty of anti-insect/herbicide transgenic plants. In addition, considerable progresses have also been made in production of drugs, pigments, food additives, enzymes, agricultural chemicals and other products with transgenic plant bioreactors.

As for animals, nuclear transplantation became successful between goldfishes and bitterlings and hybrid fishes were bred by Di-zhou Tong and others in 1973; hybridomas not only secreting monoclonal antibodies but also booming *in vitro* were successfully constructed by Kohler and Milstein in 1975, thereby the hybridoma technology was developed; the year 1977 witnessed the birth of the world's first test-tube baby in U. K. based on embryo engineering technologies; mouse embryonic stem-like cells were successfully isolated by Evans and Kanfman in 1981; the growth hormone genes of rats were transferred into mice to cultivate fast-growing super-mice by Palmiter and Brinster in 1983; sheep was cloned with embryonic cells by Danish scientist Villadsen in 1984, which was the first case of cloning mammals based on nuclear transfer; in 1987, Gordon collected transgenic mice secreting tissue plasminogen activator tPA; since then, mammary gland bioreactors for transgenic sheep, cattle, and pigs came out one after another. Clotting factors Ⅸ and Ⅷ, antitrypsin, erythropoietin, etc., have been produced with animal mammary gland bioreactors, and interferon, vaccines, monoclonal antibodies and other drugs were prepared by large scale animal cell culture. In 1987, the concept of "tissue engineering" was proposed by the National Science Foundation of United States. Cell therapy, tissue repair and artificial tissues and organs have now come to the fore with respect to combining biology with medical science. Dolly was cloned with somatic cells of adult animals in the U.K. in 1997, attesting to the totipotency of somatic cells of higher animals. Subsequently, descendants of mice, cattle and pigs were produced by cloning somatic cells. Embryonic stem lines of humans were successfully isolated and established by American scientists in 1998, which greatly contributed to stem cell researches. Recent years have witnessed significant breakthroughs in tissue engineering, stem cells, somatic cell cloning, transgenic animals and other fields, rendering cell engineering one of the forefront and hot fields in the contemporary biotechnology.

1.2.2 Relationship between Cell Engineering and other Bioengineering Technologies

The relationship between cell engineering and other bioengineering sciences is shown in Fig.1.3.