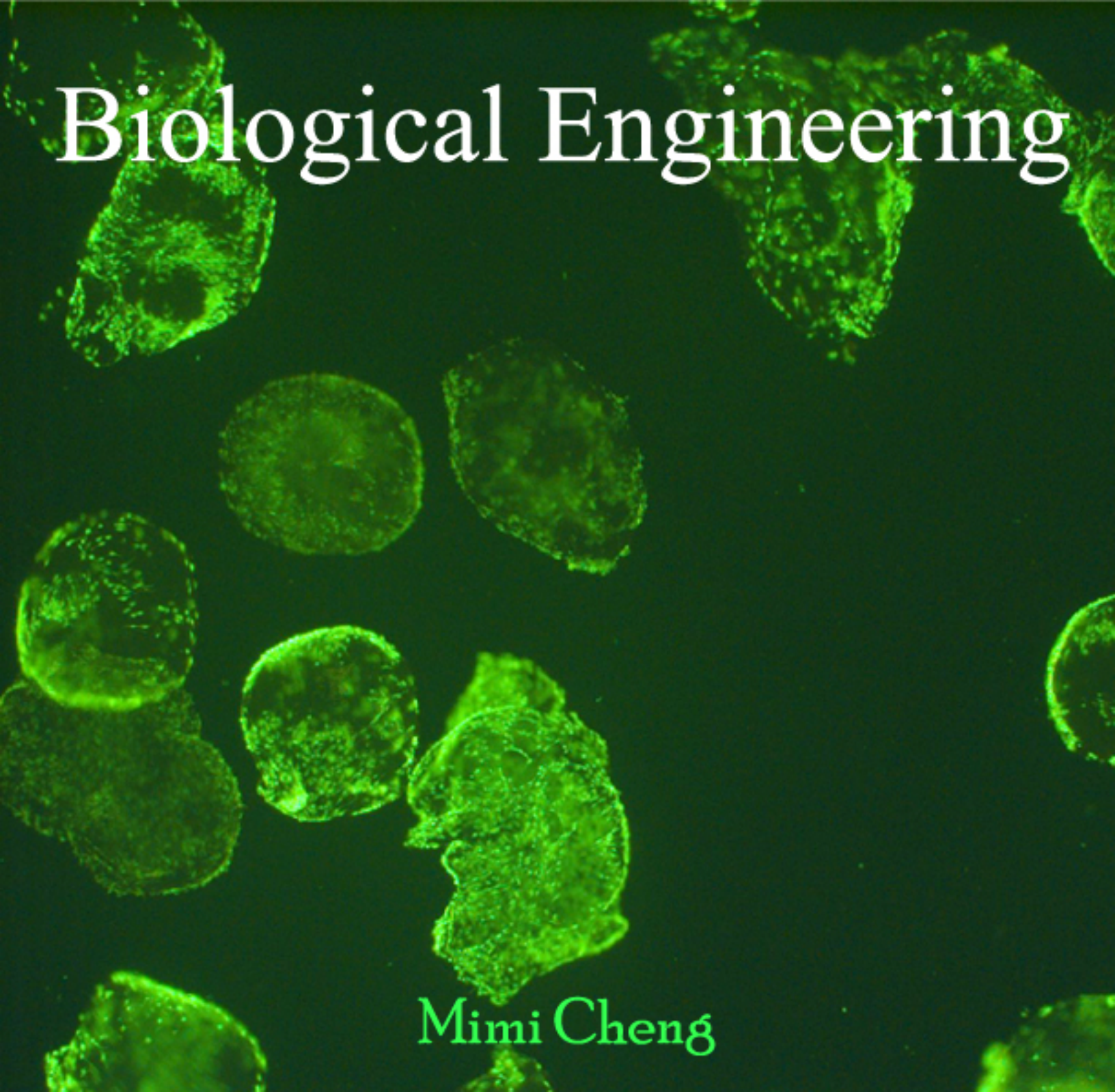
Biological Engineering



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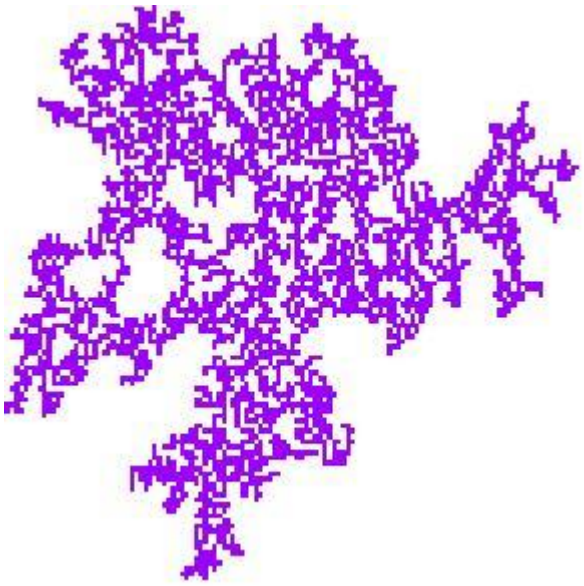


Modeling of the spread of disease using Cellular Automata and Nearest Neighbor Interactions

Biological engineering, **biotechnological engineering** or **bioengineering** (including **biological systems engineering**) is the application of concepts and methods of physics and mathematics to solve problems in life sciences, using engineering's own analytical and synthetic methodologies. In this context, while traditional engineering applies physical and mathematical sciences to analyze, design and manufacture inanimate tools, structures and processes, bioengineering uses the same sciences to study many aspects of living organisms. Usually it is used to analyze and solve problems related to human health.

Biological engineering is a science-based discipline founded upon the biological sciences in the same way that chemical engineering, electrical engineering, and mechanical engineering are based upon chemistry, electricity and magnetism and statics, respectively.

Biological engineering can be differentiated from its roots of pure biology or classical engineering in the following way. Biological studies often follow a reductionist approach in viewing a system on its smallest possible scale which naturally leads toward tools such as functional genomics. Engineering approaches, using classical design perspectives, are constructionist, building new devices, approaches, and technologies from component

concepts. Biological engineering utilizes both of these methods in concert relying on reductionist approaches to define the fundamental units which are then commingled to generate something new. Although engineered biological systems have been used to manipulate information, construct materials, process chemicals, produce energy, provide food, and help maintain or enhance human health and our environment, our ability to quickly and reliably engineer biological systems that behave as expected remains less well developed than our mastery over mechanical and electrical systems.

The differentiation between biological engineering and overlap with Biomedical Engineering can be unclear, as many universities now use the terms "bioengineering" and "biomedical engineering" interchangeably. Some contend that Biological Engineering (like biotechnology) has a broader base which spans molecular methods (tends to emphasize the using of biological substances - applying engineering principles to molecular biology, biochemistry, microbiology, pharmacology, protein chemistry, cytology, immunology, neurobiology and neuroscience, cellular and tissue based methods (including devices and sensors), whole organisms (plants, animals), and up increasing length scales to ecosystems. Neither biological engineering nor biomedical engineering is wholly contained within the other, as there are non-biological products for medical needs and biological products for non-medical needs.

ABET, the U.S.-based accreditation board for engineering B.S. programs, makes a distinction between Biomedical Engineering and Biological Engineering; however, the differences are quite small. Biomedical engineers must have life science courses that include human physiology and have experience in performing measurements on living systems while biological engineers must have life science courses (which may or may not include physiology) and experience in making measurements not specifically on living systems. Foundational engineering courses are often the same and include thermodynamics, fluid and mechanical dynamics, kinetics, electronics, and materials properties.

The word bioengineering was coined by British scientist and broadcaster Heinz Wolff in 1954. The term bioengineering is also used to describe the use of vegetation in civil engineering construction. The term bioengineering may also be applied to environmental modifications such as surface soil protection, slope stabilisation, watercourse and shoreline protection, windbreaks, vegetation barriers including noise barriers and visual screens, and the ecological enhancement of an area. The first biological engineering program was created at Mississippi State University in 1967, making it the first biological engineering curriculum in the United States. More recent programs have been launched at MIT and Utah State University.

Biological Engineers or *bioengineers* are engineers who use the principles of biology and the tools of engineering to create usable, tangible products. Biological Engineering employs knowledge and expertise from a number of pure and applied sciences, such as mass and heat transfer, kinetics, biocatalysts, biomechanics, bioinformatics, separation and purification processes, bioreactor design, surface science, fluid mechanics, thermodynamics, and polymer science. It is used in the design of medical devices,

diagnostic equipment, biocompatible materials, renewable bioenergy, ecological engineering, and other areas that improve the living standards of societies.

In general, biological engineers attempt to either mimic biological systems in order to create products or modify and control biological systems so that they can replace, augment, or sustain chemical and mechanical processes. Bioengineers can apply their expertise to other applications of engineering and biotechnology, including genetic modification of plants and microorganisms, bioprocess engineering, and biocatalysis.

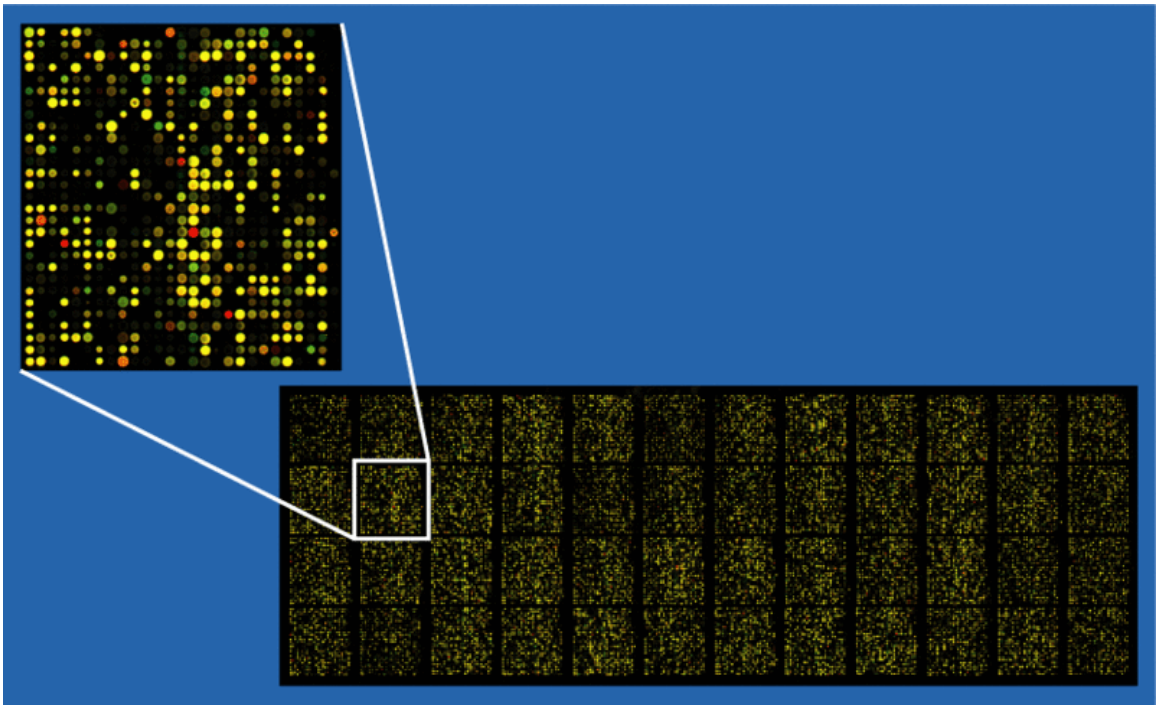
Because other engineering disciplines also address living organisms (e.g., prosthetics in mechanical engineering), the term biological engineering can be applied more broadly to include agricultural engineering and biotechnology. In fact, many old agricultural engineering departments in universities over the world have rebranded themselves as **agricultural and biological engineering** or **agricultural and biosystems engineering**. Biological engineering is also called bioengineering by some colleges and Biomedical engineering is called Bioengineering by others, and is a rapidly developing field with fluid categorization. The Main Fields of Bioengineering may be categorised as:

- **Bioprocess Engineering**: Bioprocess Design, Biocatalysis, Bioseparation, Bioinformatics, Bioenergy
- **Genetic Engineering**: Synthetic Biology, Horizontal gene transfer.
- **Cellular Engineering**: Cell Engineering, Tissue Culture Engineering, Metabolic engineering.
- **Biomedical Engineering**: Biomedical technology, Biomedical Diagnostics, Biomedical Therapy, Biomechanics, Biomaterials.

Chapter- 1

Biomedical Engineering

A JARVIK-7 artificial heart, an example of a biomedical engineering application of mechanical engineering with biocompatible materials for cardiothoracic surgery using an artificial organ.



Example of an approximately 40,000 probe spotted oligo microarray with enlarged inset to show detail.

Biomedical engineering is the application of engineering principles and techniques to the medical field. This field seeks to close the gap between **engineering** and **medicine**: It combines the design and problem solving skills of engineering with medical and biological sciences to improve healthcare diagnosis, monitoring and therapy.

Biomedical engineering has only recently emerged as its own discipline, compared to many other engineering fields; such an evolution is common as a new field transitions from being an interdisciplinary specialization among already-established fields, to being considered a field in itself. Much of the work in biomedical engineering consists of research and development, spanning a broad array of subfields (see below). Prominent biomedical engineering applications include the development of biocompatible prostheses, various diagnostic and therapeutic medical devices ranging from clinical equipment to micro-implants, common imaging equipment such as MRIs and EEGs, biotechnologies such as regenerative tissue growth, and pharmaceutical drugs and biopharmaceuticals.

Subdisciplines within biomedical engineering

Biomedical engineering is a highly interdisciplinary field, influenced by (and overlapping with) various other engineering and medical fields. This often happens with newer disciplines, as they gradually emerge in their own right after evolving from special applications of extant disciplines. Due to this diversity, it is typical for a biomedical engineer to focus on a particular subfield or group of related subfields. There are many different taxonomic breakdowns within BME, as well as varying views about how best to

organize them and manage any internal overlap; the main U.S. organization devoted to BME divides the major specialty areas as follows:

- Biomechatronics
- Bioinstrumentation
- Biomaterials
- Biomechanics
- Bionics
- Cellular, Tissue, and Genetic Engineering
- Clinical Engineering
- Medical Imaging
- Orthopaedic Bioengineering
- Rehabilitation engineering
- Systems Physiology
- Bionanotechnology
- Neural Engineering

Sometimes, disciplines within BME are classified by their association(s) with other, more established engineering fields, which can include:

- Chemical engineering - often associated with biochemical, cellular, molecular and tissue engineering, biomaterials, and biotransport.
- Electrical engineering - often associated with bioelectrical and neural engineering, bioinstrumentation, biomedical imaging, and medical devices. This also tends to encompass Optics and Optical engineering - biomedical optics, imaging and related medical devices.
- Mechanical engineering - often associated with biomechanics, biotransport, medical devices, and modeling of biological systems, like soft tissue mechanics.

Biotechnology and pharmaceuticals

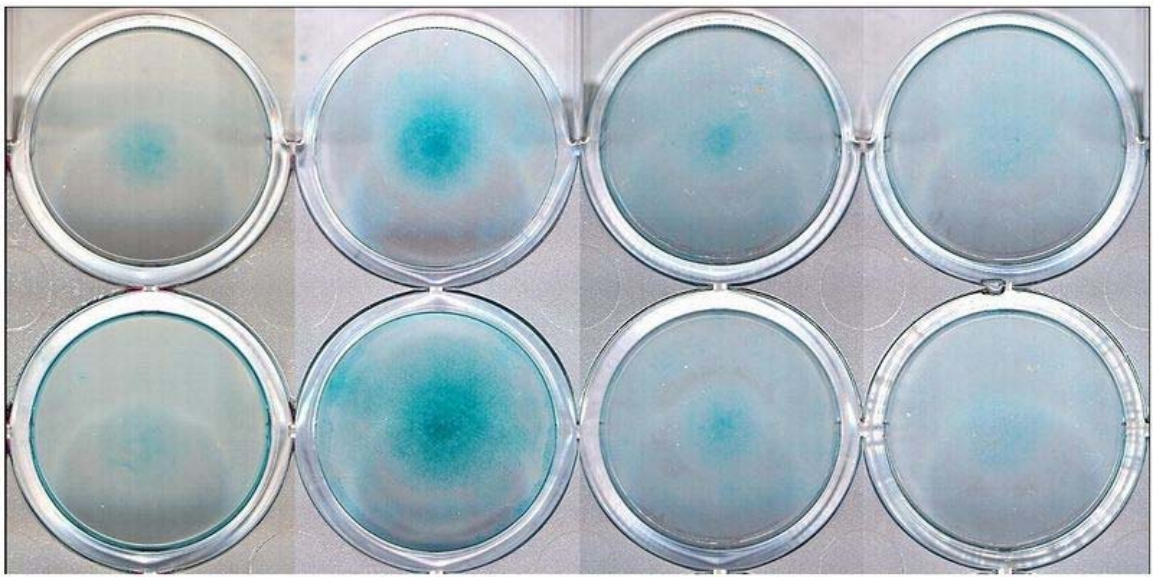
Biotechnology can be a somewhat ambiguous term, sometimes loosely used interchangeably with BME in general; however, it more typically denotes specific products which use "biological systems, living organisms, or derivatives thereof." Even some complex "medical devices" (see below) can reasonably be deemed "biotechnology" depending on the degree to which such elements are central to their principle of operation. Biologics/Biopharmaceuticals (e.g., vaccines, stored blood product), genetic engineering, and various agricultural applications are some major classes of biotechnology.

Pharmaceuticals are related to biotechnology in two indirect ways: 1) certain major types (e.g. biologics) fall under both categories, and 2) together they essentially comprise the "*non*-medical-device" set of BME applications. (The "Device - Bio/Chemical" spectrum is an imperfect dichotomy, but one regulators often use, at least as a starting point.)

Tissue engineering

Tissue engineering is a major segment of *Biotechnology*.

One of the goals of tissue engineering is to create artificial organs (via biological material) for patients that need organ transplants. Biomedical engineers are currently researching methods of creating such organs. Researchers have grown solid jawbones and tracheas from human stem cells towards this end. Several artificial urinary bladders actually have been grown in laboratories and transplanted successfully into human patients. Bioartificial organs, which use both synthetic and biological components, are also a focus area in research, such as with hepatic assist devices that use liver cells within an artificial bioreactor construct.



Micromass cultures of C3H-10T1/2 cells at varied oxygen tensions stained with Alcian blue.

Genetic Engineering

Genetic engineering, recombinant DNA technology, genetic modification/manipulation (GM) and gene splicing are terms that apply to the direct manipulation of an organism's genes. Genetic engineering is different from traditional breeding, where the organism's genes are manipulated indirectly. Genetic engineering uses the techniques of molecular cloning and transformation to alter the structure and characteristics of genes directly. Genetic engineering techniques have found success in numerous applications. Some examples are in improving crop technology, the manufacture of synthetic human insulin through the use of modified bacteria, the manufacture of erythropoietin in hamster ovary cells, and the production of new types of experimental mice such as the oncomouse (cancer mouse) for research.

Neural Engineering

Neural engineering (also known as Neuroengineering) is a discipline that uses engineering techniques to understand, repair, replace, or enhance neural systems. Neural engineers are uniquely qualified to solve design problems at the interface of living neural tissue and non-living constructs.

Pharmaceutical engineering

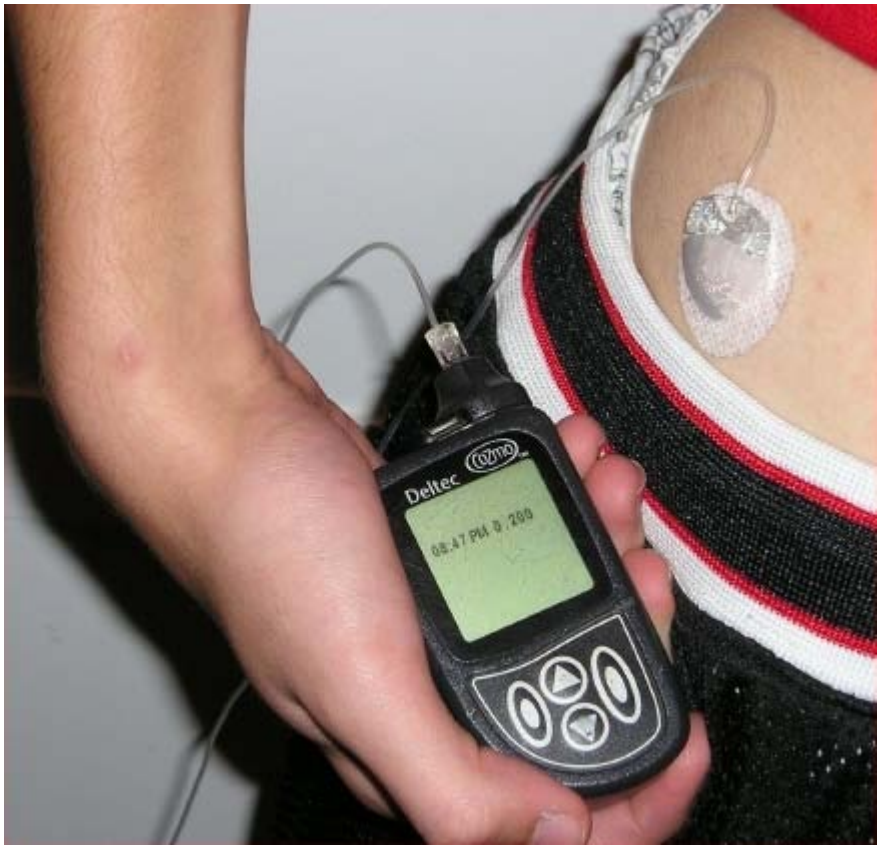
Pharmaceutical Engineering is sometimes regarded as a branch of biomedical engineering, and sometimes a branch of chemical engineering; in practice, it is very much a hybrid sub-discipline (as many BME fields are). Aside from those pharmaceutical products directly incorporating biological agents or materials, even developing chemical drugs is considered to require substantial BME knowledge due to the physiological interactions inherent to such products' usage.

Medical devices

This is an *extremely broad category* -- essentially covering all health care products that do **not** achieve their intended results through predominantly chemical (e.g., pharmaceuticals) or biological (e.g., vaccines) means, and do not involve metabolism.

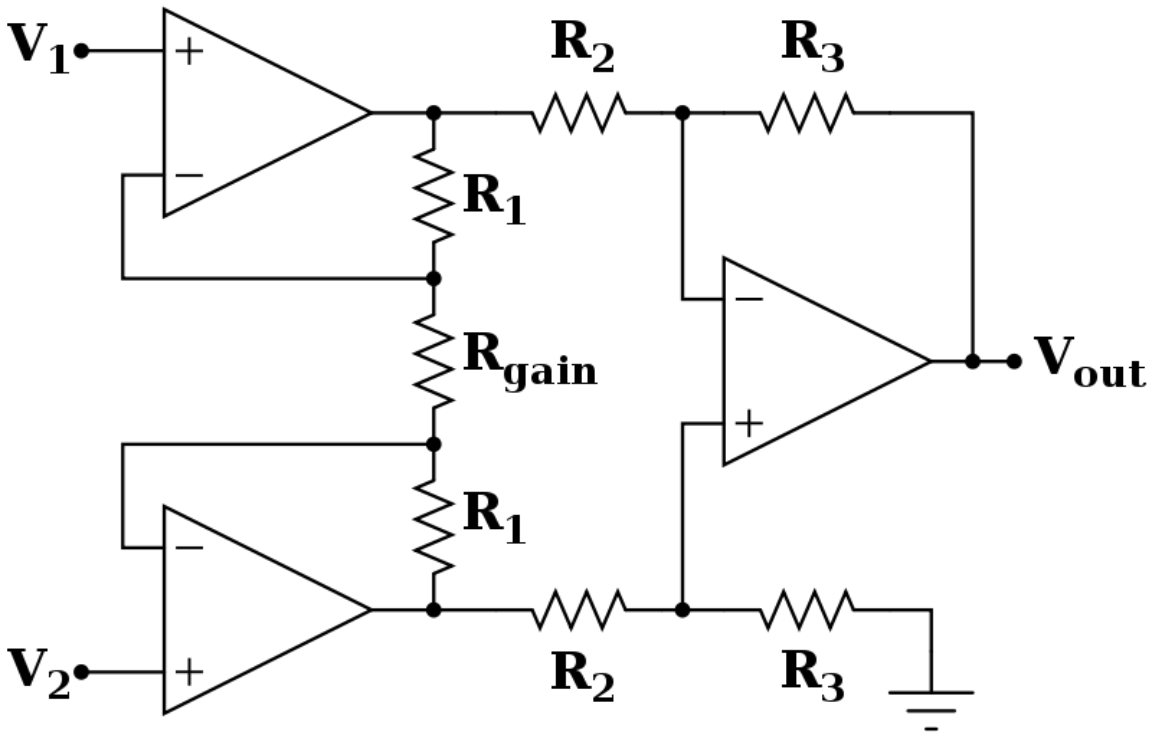
A medical device is intended for use in:

- the diagnosis of disease or other conditions, or
- in the cure, mitigation, treatment, or prevention of disease,



A pump for continuous subcutaneous insulin infusion, an example of a biomedical engineering application of electrical engineering to medical equipment.

Some examples include pacemakers, infusion pumps, the heart-lung machine, dialysis machines, artificial organs, implants, artificial limbs, corrective lenses, cochlear implants, ocular prosthetics, facial prosthetics, somato prosthetics, and dental implants.



Biomedical instrumentation amplifier schematic used in monitoring low voltage biological signals, an example of a biomedical engineering application of electronic engineering to electrophysiology.

Stereolithography is a practical example of *medical modeling* being used to create physical objects. Beyond modeling organs and the human body, emerging engineering techniques are also currently used in the research and development of new devices for innovative therapies, treatments, patient monitoring, and early diagnosis of complex diseases.

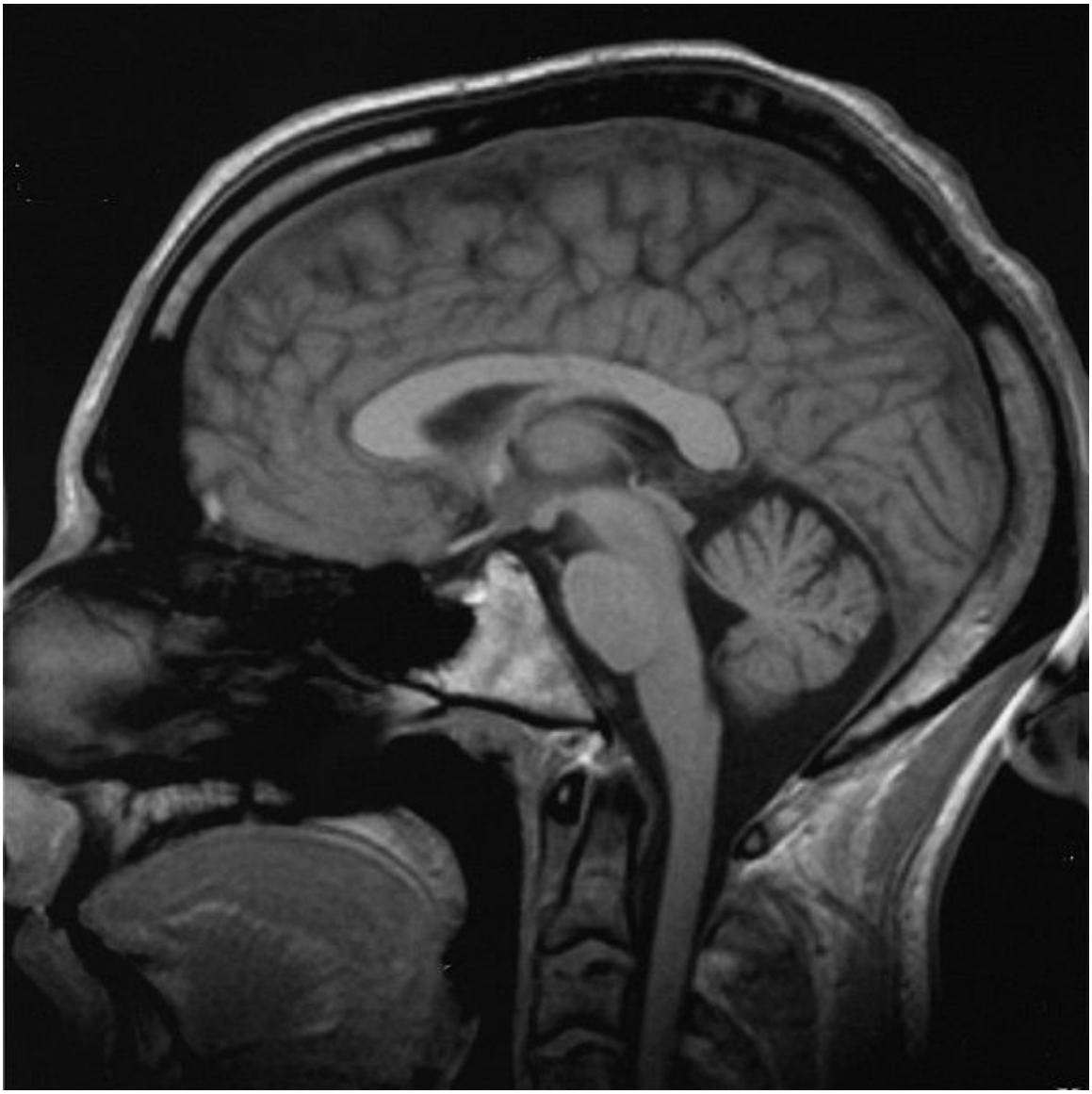
Medical devices are regulated and classified (in the US) as follows:

1. Class I devices present minimal potential for harm to the user and are often simpler in design than Class II or Class III devices. Devices in this category include tongue depressors, bedpans, elastic bandages, examination gloves, and hand-held surgical instruments and other similar types of common equipment.
2. Class II devices are subject to special controls in addition to the general controls of Class I devices. Special controls may include special labeling requirements, mandatory performance standards, and postmarket surveillance. Devices in this class are typically non-invasive and include x-ray machines, PACS, powered wheelchairs, infusion pumps, and surgical drapes.
3. Class III devices generally require premarket approval (PMA) or premarket notification (510k), a scientific review to ensure the device's safety and effectiveness, in addition to the general controls of Class I. Examples include replacement heart valves, hip and knee joint implants, silicone gel-filled breast

implants, implanted cerebellar stimulators, implantable pacemaker pulse generators and endosseous (intra-bone) implants.

Medical imaging

Medical/biomedical imaging is a major segment of medical devices. This area deals with enabling clinicians to directly or indirectly "view" things not visible in plain sight (such as due to their size, and/or location). This can involve utilizing ultrasound, magnetism, UV, other radiology, and other means.



An MRI scan of a human head, an example of a biomedical engineering application of electrical engineering to diagnostic imaging.

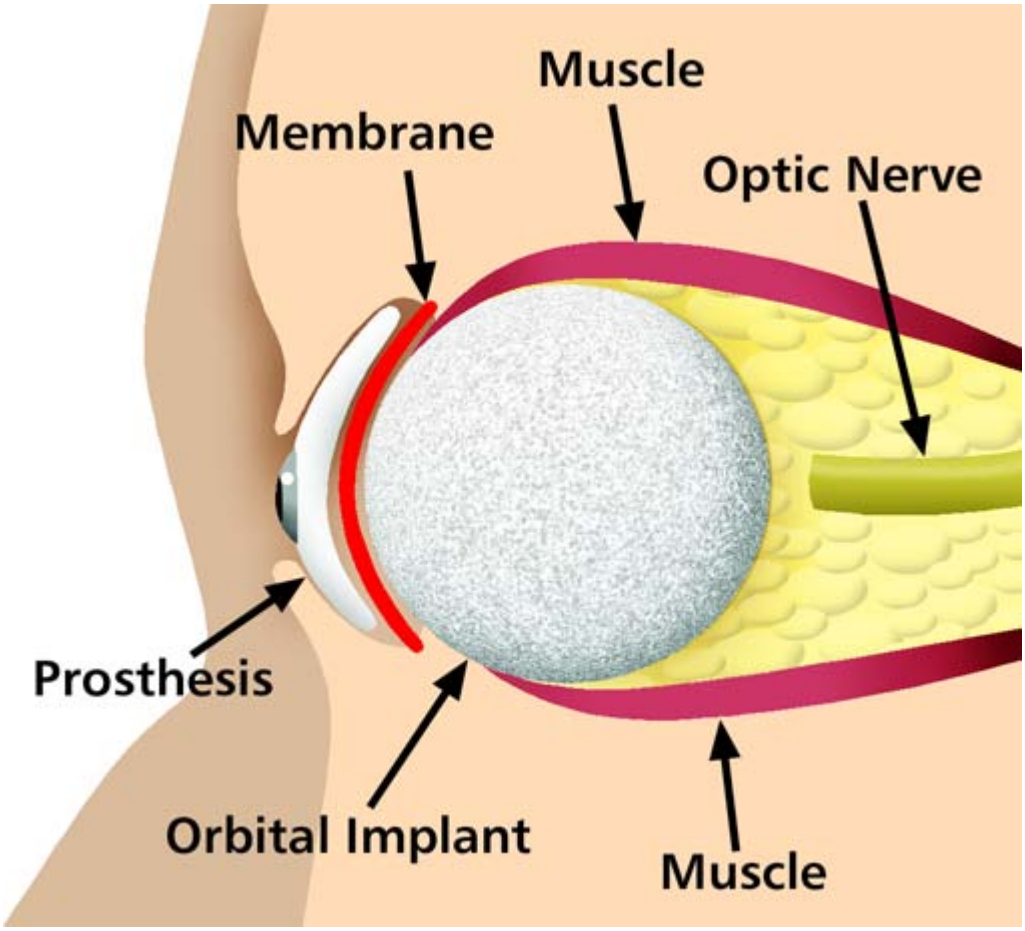
Imaging technologies are often essential to medical diagnosis, and are typically the most complex equipment found in a hospital including:

- Fluoroscopy
- Magnetic resonance imaging (MRI)
- Nuclear medicine
- Positron emission tomography (PET) PET scansPET-CT scans
- Projection radiography such as X-rays and CT scans
- Tomography
- Ultrasound
- Optical microscopy
- Electron microscopy

Implants

An implant is a kind of medical device made to replace and act as a missing biological structure (as compared with a transplant, which indicates transplanted biomedical tissue). The surface of implants that contact the body might be made of a biomedical material such as titanium, silicone or apatite depending on what is the most functional. In some cases implants contain electronics e.g. artificial pacemaker and cochlear implants. Some implants are bioactive, such as subcutaneous drug delivery devices in the form of implantable pills or drug-eluting stents.

Artificial limbs: The right arm is an example of a prosthesis, and the left arm is an example of myoelectric control.



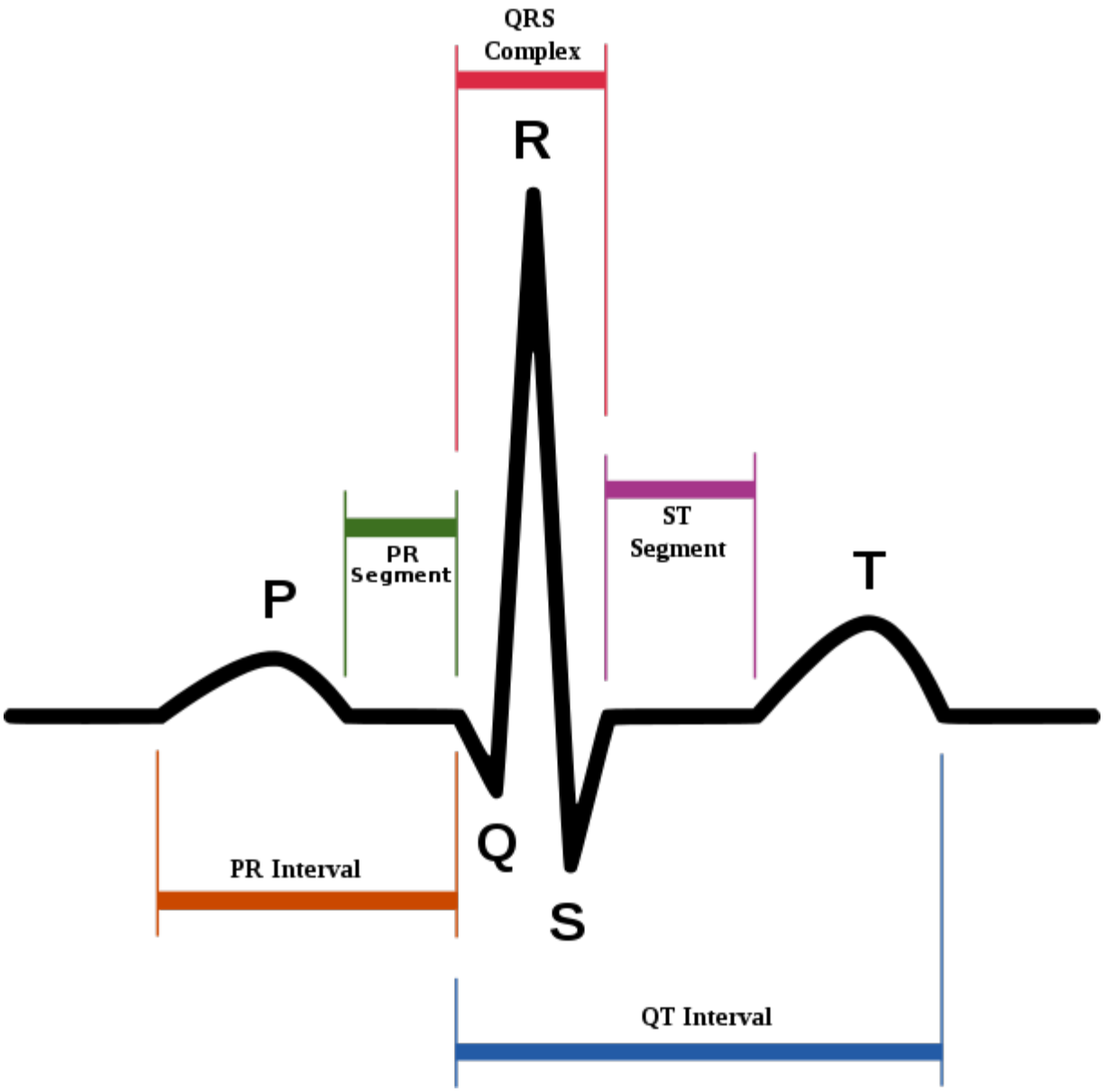
A prosthetic eye, an example of a biomedical engineering application of mechanical engineering and biocompatible materials to ophthalmology.

Clinical engineering

Clinical engineering is the branch of biomedical engineering dealing with the actual implementation of medical equipment and technologies in hospitals or other clinical settings. Major roles of clinical engineers include training and supervising biomedical equipment technicians (BMETs), selecting technological products/services and logistically managing their implementation, working with governmental regulators on inspections/audits, and serving as technological consultants for other hospital staff (e.g. physicians, administrators, I.T., etc.). Clinical engineers also advise and collaborate with medical device producers regarding prospective design improvements based on clinical experiences, as well as monitor the progression of the state-of-the-art so as to redirect procurement patterns accordingly.

Their inherent focus on *practical* implementation of technology has tended to keep them oriented more towards *incremental*-level redesigns and reconfigurations, as opposed to revolutionary research & development or ideas that would be many years from clinical adoption; however, there is a growing effort to expand this time-horizon over which clinical engineers can influence the trajectory of biomedical innovation. In their various

roles, they form a "bridge" between the primary designers and the end-users, by combining the perspectives of being both 1) close to the point-of-use, while 2) trained in product and process engineering. Clinical Engineering departments will sometimes hire not just biomedical engineers, but also industrial/systems engineers to help address operations research/optimization, human factors, cost analysis, etc.



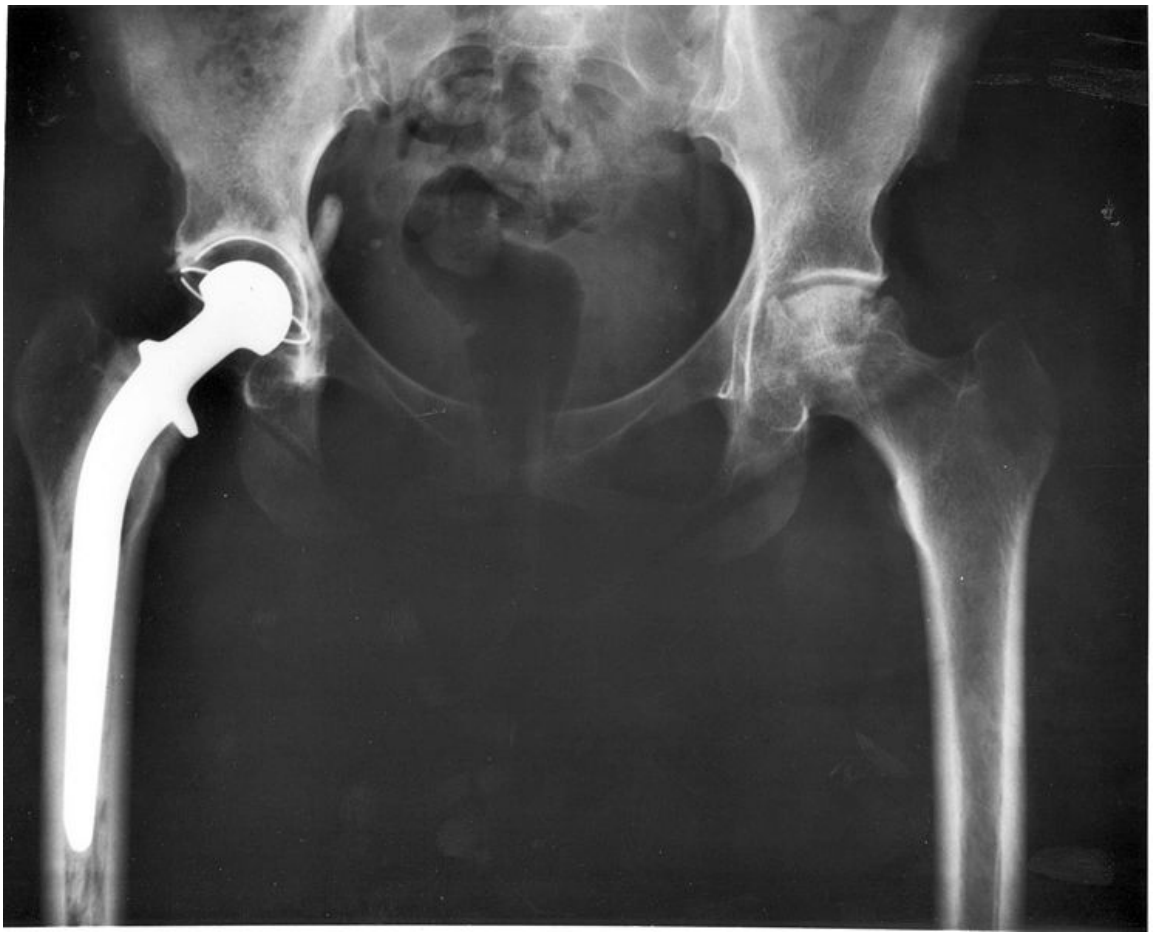
Schematic representation of a normal ECG trace showing *sinus rhythm;* an example of widely-used clinical medical equipment (operates by applying electronic engineering to electrophysiology and medical diagnosis.

A point of reference for clinical engineers would be the catalogue published by The American Society for Hospital Engineering in the Hospital Engineering Reference Series called Maintenance Management for Medical Equipment

Regulatory issues

Regulatory issues are of particular concern to a biomedical engineer; it is among the most heavily-regulated fields of engineering, and practicing biomedical engineers must routinely consult and cooperate with regulatory law attorneys and other experts. The Food and Drug Administration (FDA) is the principal healthcare regulatory authority in the United States, having jurisdiction over medical *devices, drugs, biologics, and combination* products. The paramount objectives driving policy decisions by the FDA are **safety** and **efficacy** of healthcare products.

In addition, because biomedical engineers often develop devices and technologies for "consumer" use, such as physical therapy devices (which are also "medical" devices), these may also be governed in some respects by the Consumer Product Safety Commission. The greatest hurdles tend to be 510K "clearance" (typically for Class 2 devices) or pre-market "approval" (typically for drugs and class 3 devices).



Implants, such as artificial hip joints, are generally extensively regulated due to the invasive nature of such devices.

Most countries have their own particular mechanisms for regulation, with varying formulations and degrees of restrictiveness. In most European countries, more discretion

rests with the prescribing doctor, while the regulations chiefly assure that the product operates as expected. In European Union nations, the national governments license certifying agencies, which are for-profit companies. Technical committees of engineers write recommendations which incorporate public comments, and these can be adopted as regulations by the European Union. These recommendations vary by the type of device, and specify tests for safety and efficacy. Once a prototype has passed the tests at a certification lab, and that model is being constructed under the control of a certified quality system, the device is entitled to bear a CE mark, indicating that the device is believed to be safe and reliable when used as directed.

The different regulatory arrangements sometimes result in particular technologies being developed first for either the U.S. or in Europe depending on the more favorable form of regulation. While nations often strive for substantive harmony to facilitate cross-national distribution, philosophical differences about the *optimal extent* of regulation can be a hindrance; more restrictive regulations seem appealing on an intuitive level, but critics decry the tradeoff cost in terms of slowing access to life-saving developments.

Training and certification

Education

Biomedical engineers require considerable knowledge of both engineering and biology, and typically have a Master's (M.S., M.S.E., or M.Eng.) or a Doctoral (Ph.D.) degree in BME or another branch of engineering with considerable potential for BME overlap. As interest in BME is increasing, many engineering colleges now have a Biomedical Engineering Department or Program, with offerings ranging from the undergraduate (B.S. or B.S.E.) to the doctoral levels. As noted above, biomedical engineering has only recently been emerging as *its own discipline* rather than a cross-disciplinary hybrid specialization of other disciplines; now, BME programs of study at all levels are becoming more widespread, including the Bachelor of Science in Biomedical Engineering which actually includes so much biological science content that many students use it as a "pre-med" major in preparation for medical school. The number of biomedical engineers is expected to rise as both a cause and effect of improvements in medical technology.

In the U.S., an increasing number of undergraduate programs are also becoming recognized by ABET as accredited bioengineering/biomedical engineering programs. Over 65 programs are currently accredited by ABET.

As with many degrees, the reputation and ranking of a program may factor into the desirability of a degree holder for either employment or graduate admission. The reputation of many undergraduate degrees are also linked to the institution's graduate or research programs, which have some tangible factors for rating, such as research funding and volume, publications and citations. With BME specifically, the ranking of a university's hospital and medical school can also be a significant factor in the perceived prestige of its BME department/program.

Graduate education is a particularly important aspect in BME. While many engineering fields (such as mechanical or electrical engineering) do not need graduate-level training to obtain an entry-level job in their field, the majority of BME positions do prefer or even require them. Since most BME-related professions involve scientific research, such as in pharmaceutical and medical device development, graduate education is almost a requirement (as undergraduate degrees typically do not involve sufficient research training and experience). This can be either a **Masters** or **Doctoral** level degree; while in certain specialties a Ph.D. is notably more common than in others, it is hardly ever the majority (except in academia). In fact, the perceived need for some kind of graduate credential is so strong that some undergraduate BME programs will actively discourage students from majoring in BME without an expressed intention to also obtain a masters degree or apply to medical school afterwards.

Graduate programs in BME, like in other scientific fields, are highly varied, and particular programs may emphasize certain aspects within the field. They may also feature extensive collaborative efforts with programs in other fields (such as the University's Medical School or other engineering divisions), owing again to the interdisciplinary nature of BME. M.S. and Ph.D. programs will typically require applicants to have an undergraduate degree in BME, or *another engineering* discipline (plus certain life science coursework), or *life science* (plus certain engineering coursework).

Education in BME also varies greatly around the world. By virtue of its extensive biotechnology sector, its numerous major universities, and relatively few internal barriers, the U.S. has progressed a great deal in its development of BME education and training opportunities. Europe, which also has a large biotechnology sector and an impressive education system, has encountered trouble in creating uniform standards as the European community attempts to supplant some of the national jurisdictional barriers that still exist. Recently, initiatives such as BIOMEDEA have sprung up to develop BME-related education and professional standards. Other countries, such as Australia, are recognizing and moving to correct deficiencies in their BME education. Also, as high technology endeavors are usually marks of developed nations, some areas of the world are prone to slower development in education, including in BME.

Licensure/Certification

Engineering licensure in the US is largely optional, and rarely specified by branch/discipline. As with other learned professions, each state has certain (fairly similar) requirements for becoming licensed as a registered professional engineer (PE), but in practice such a license is not required to practice in the majority of situations (due to an exception known as the private industry exemption, which effectively applies to the vast majority of American engineers). This is notably not the case in many other countries, where a license is as legally necessary to practice engineering as it is for law or medicine.

Biomedical engineering is regulated in some countries, such as Australia, but registration is typically only recommended and not required.

In the UK, mechanical engineers working in the areas of Medical Engineering, Bioengineering or Biomedical engineering can gain Chartered Engineer status through the Institution of Mechanical Engineers. The Institution also runs the Engineering in Medicine and Health Division.

The Fundamentals of Engineering exam - the first (and more general) of two licensure examinations for most U.S. jurisdictions—does now cover biology (although technically not BME). For the second exam, called Part 2 or the Professional Engineering exam, candidates may select a particular engineering discipline's content to be tested on; there is currently not an option for BME with this, meaning that any biomedical engineers seeking a license must prepare to take this examination in another category (which does not affect the actual license, since most jurisdictions do not recognize discipline specialties anyway). However, the Biomedical Engineering Society (BMES) is, as of 2009, exploring the possibility of seeking to implement a BME-specific version of this exam to facilitate biomedical engineers pursuing licensure.

Beyond governmental registration, certain private-sector professional/industrial organizations also offer certifications with varying degrees of prominence. One such example is the Certified Clinical Engineer (CCE) certification for Clinical engineers.

Founding figures

- Leslie Geddes (deceased)- Professor Emeritus at Purdue University, electrical engineer, inventor and educator of over 2000 biomedical engineers, received a National Medal of Technology in 2006 from President George Bush for his more than 50 years of contributions that have spawned innovations ranging from burn treatments to miniature defibrillators, ligament repair to tiny blood pressure monitors for premature infants, as well as a new method for performing cardiopulmonary resuscitation (CPR).
- Y. C. Fung - professor emeritus at the University of California, San Diego, considered by many to be the founder of modern Biomechanics
- Robert Langer - Institute Professor at MIT, runs the largest BME laboratory in the world, pioneer in drug delivery and tissue engineering
- Herbert Lissner (deceased) - Professor of Engineering Mechanics at Wayne State University. Initiated studies on blunt head trauma and injury thresholds beginning in 1939 in collaboration with Dr. E.S. Gurdjian, a neurosurgeon at Wayne State's School of Medicine. Individual for whom the American Society of Mechanical Engineers' top award in Biomedical Engineering, the Herbert R. Lissner Medal, is named.
- Nicholas A. Peppas - Chaired Professor in Engineering, University of Texas at Austin, pioneer in drug delivery, biomaterials, hydrogels and nanobiotechnology.
- Otto Schmitt (deceased) - biophysicist with significant contributions to BME, working with biomimetics
- Ascher Shapiro (deceased) - Institute Professor at MIT, contributed to the development of the BME field, medical devices (e.g. intra-aortic balloons)

- John G. Webster - Professor Emeritus at the University of Wisconsin–Madison, a pioneer in the field of instrumentation amplifiers for the recording of electrophysiological signals
- Robert Plonsey - Professor Emeritus at Duke University, pioneer of electrophysiology
- U. A. Whitaker (deceased) - provider of The Whitaker Foundation, which supported research and education in BME by providing over $700 million to various universities, helping to create 30 BME programs and helping finance the construction of 13 buildings
- Frederick Thurstone (deceased) - Professor Emeritus at Duke University, pioneer of diagnostic ultrasound
- Alfred E. Mann - Physicist, entrepreneur and philanthropist. A pioneer in the field of Biomedical Engineering.
- Forrest Bird - aviator and pioneer in the invention of mechanical ventilators
- Willem Johan Kolff (deceased) - pioneer of hemodialysis as well as in the field of artificial organs
- Medical device

Chapter- 2

Clinical Engineering

Clinical engineering is a specialty within Biomedical engineering responsible primarily for applying and implementing medical technology to optimize healthcare delivery. Roles of clinical engineers include training and supervising biomedical equipment technicians (BMETs), working with governmental regulators on hospital inspections/audits, and serving as technological consultants for other hospital staff (i.e. physicians, administrators, I.T., etc.). Clinical engineers also advise medical device producers regarding prospective design improvements based on clinical experiences, as well as monitor the progression of the state-of-the-art in order to redirect hospital procurement patterns accordingly.

Their inherent focus on *practical* implementation of technology has tended to keep them oriented more towards *incremental*-level redesigns and reconfigurations, as opposed to "revolutionary" R&D or cutting-edge ideas that would be many years from clinical adoptability; however, there is nonetheless an effort to expand this time-horizon over which clinical engineers can influence the trajectory of biomedical innovation. In their various roles, they form a sort of "bridge" between product originators and end-users, by combining the perspectives of being both close to the point-of-use ("front lines"), while also trained in product and process design. Clinical Engineering departments at large hospitals will sometimes hire not just biomedical engineers, but also industrial/systems engineers to help address operations research, human factors, cost analyses, safety, etc.

History

While some trace its roots back to the 1940s, the actual term "clinical engineering" was first used in 1969. The first explicit published reference to the term "clinical engineering" appears in a paper published in 1969 by Landoll and Caceres . Cesar A. Caceres, a cardiologist, is generally credited with coining the term "clinical engineering." Of course, the broader field of "biomedical engineering" has a relatively recent history as well. The first modern professional intersociety engineering meeting to be focused on the application of engineering in medicine was probably held in 1948, according to the Alliance for Engineering in Medicine and Biology

The general notion of the application of engineering to medicine can be traced back centuries; for example, Stephen Hales's work in the early 18th century which led to the

invention of a ventilator and the discovery of blood pressure certainly involved the application of engineering techniques to medicine .

The recent history of this sub-discipline is somewhat erratic. In the early 1970s, clinical engineering was thought to be a field that would require many new professionals. Estimates for the US ranged as high as 5,000 to 8,000 clinical engineers, or five to ten clinical engineers for every 250,000 of population, or one clinical engineer per 250 hospital beds. . However, even then, only 300 to 400 clinical engineers had found employment in hospitals. The following decades have shown no evidence of growth in the number of employed clinical engineers, and probable evidence of decline. Nonetheless, some large hospitals do hire several clinical engineers, and some academic programs do concentrate in it.

The history of its formal credentialization and accreditation procedures has also been somewhat unstable. The International Certification Commission for Clinical Engineers (ICC) was formed under the sponsorship of the Association for the Advancement of Medical Instrumentation (AAMI) in the early 1970s, to provide a formal certification process for clinical engineers. A similar certification program was formed by academic institutions offering graduate degrees in clinical engineering as the American Board of Clinical Engineering (ABCE). In 1979, the ABCE agreed to dissolve, and those certified under its program were accepted into the ICC certification program. By 1985, only 350 clinical engineers had become certified . Finally, in 1999, AAMI after lengthy deliberation, and analysis of a 1998 survey demonstrating that there was not a viable market for its certification program decided to suspend that program, no longer accepting any new applicants as of July 1999 .

The new, current Clinical Engineering Certification (CCE) program was started in 2002 under the sponsorship of the American College of Clinical Engineering (ACCE), and is administered by the ACCE Healthcare Technology Foundation. In 2004, the first year that the certification process was actually underway, 112 individuals were granted certification based upon their previous ICC certification, and three individuals were awarded the new certification. By the time of the publication of the 2006-2007 AHTF Annual Report (approx. June 30, 2007), a total of 147 individuals were included in the ranks of HTF certified clinical engineers.

Clinical engineering in India

Healthcare has increasingly become technology driven and requires trained manpower to keep pace with the growing demand for professionals in the field. An M-Tech Clinical Engineering course was initiated by Indian Institute of Technology Madras (IITM), Sree Chitra Tirunal Institute of Medical Sciences and Technology, Trivandrum and Christian Medical College, Vellore (CMC), to address the country's need of human resource development. This was aimed for indigenous Biomedical Device Development as well as Technology management, and thereby contribute to the overall development of healthcare delivery in the country. During the course, students of engineering are given an insight into biology, medicine, relevant electronic background, clinical practices,

device development and even management aspects. Additionally, students are paired with clinical doctors from CMC and SCTIMST to get hands-on experience during internships. An important aspect of this training is simultaneous, long term and detailed exposure to clinical environment as well as to medical device development activity. This is aimed at making students understand the process of identifying 'unmet clinical need' and thus, contributing to the development of new medical devices in the country. A unique feature of the course is clinical attachment which exposes the students to the clinical environment. The program also trains engineers to manage and ensure safe and effective use of technology in heath care delivery points. The minimum essential qualification for joining this course is bachelors degree in any discipline of engineering except civil engineering and a valid GATE score in their respective fields.

The Definition

A **Clinical engineer** is defined by ACCE as "a professional who supports and advances patient care by applying engineering and managerial skills to healthcare technology." This definition was first adopted by the ACCE Board of Directors on May 13, 1991. Clinical Engineering is also recognized by the Biomedical Engineering Society (BMES), the major professional organization for biomedical engineering, as being a branch within Biomedical Engineering.

There are at least two issues with the ACCE definition that cause some confusion. First, it is phrased so broadly that it's not readily evident that "clinical engineer" is but one subset of "biomedical engineer." Many times the terms actually get used interchangeably: some hospitals refer to their relevant departments as "Clinical Engineering" departments, while others call them "Biomedical Engineering" departments. Indeed, as noted above, the *technicians* are almost universally referred to as "biomedical equipment technicians," regardless of the name of the department that they might work under. However, the term "biomedical engineer" is generally thought to be more all-encompassing, including engineers who work in the primary design of medical devices for manufacturers, or in original R&D, or in academia—whereas clinical engineers generally work in hospitals solving problems that are very close to where equipment is actually used in a patient care setting. The clinical engineers in some countries such as India are trained to innovate and find technological solutions for the clinical needs. . The other issue not evident from the ACCE definition is the appropriate educational background for a clinical engineer. Generally, the expectation of the certification program is that an applicant for certification as a clinical engineer will hold an accredited bachelor's degree in engineering (or at least engineering technology).

The future

The management of healthcare technology is becoming increasingly complex. The driving factors and opportunities presented are examined in The Future of Clinical Engineering, published in the IEEE EMBS magazine in 2003.

Eligibility Requirements

To be eligible for certification in clinical engineering (CCE), a candidate must hold appropriate professional or educational credentials (an accredited engineering or possibly engineering-technology degree) have certain relevant experience, and pass an examination. The Examination for Certification in Clinical Engineering involves a written examination composed of a maximum of 150 multiple-choice objective questions with a testing time of three (3) hours, and a separate oral exam. . Particular weight is given to applicants for CE certification (CCE) who are already licensed as registered Professional Engineers (PE) -- which itself has extensive requirements (including an accredited engineering degree and engineering experience).

Chapter- 3

Genetic Engineering

Genetic engineering, also called **genetic modification**, is the direct human manipulation of an organism's genetic material in a way that does not occur under natural conditions. It involves the use of recombinant DNA techniques, but does not include traditional animal and plant breeding or mutagenesis. Any organism that is generated using these techniques is considered to be a genetically modified organism. The first organisms genetically engineered were bacteria in 1973 and then mice in 1974. Insulin producing bacteria were commercialized in 1982 and genetically modified food has been sold since 1994.

The most common form of genetic engineering involves the insertion of new genetic material at an unspecified location in the host genome. This is accomplished by isolating and copying the genetic material of interest, generating a construct containing all the genetic elements for correct expression, and then inserting this construct into the host organism. Other forms of genetic engineering include gene targeting and knocking out specific genes via engineered nucleases such as zinc finger nucleases or engineered homing endonucleases.

Genetic engineering techniques have been applied in numerous fields including research, biotechnology, and medicine. Medicines such as insulin and human growth hormone are now produced in bacteria, experimental mice such as the oncomouse and the knockout mouse are being used for research purposes and insect resistant and/or herbicide tolerant crops have been commercialized. Genetically engineered plants and animals capable of producing biotechnology drugs more cheaply than current methods (called pharming) are also being developed and in 2009 the FDA approved the sale of the pharmaceutical protein antithrombin produced in the milk of genetically engineered goats.

Definition

Genetic engineering alters the genetic makeup of an organism using techniques that introduce heritable material prepared outside the organism either directly into the host or into a cell that is then fused or hybridized with the host. This involves using recombinant nucleic acid (DNA or RNA) techniques to form new combinations of heritable genetic material followed by the incorporation of that material either indirectly through a vector system or directly through micro-injection, macro-injection and micro-encapsulation techniques. Genetic engineering does not include traditional animal and plant breeding, in

vitro fertilisation, induction of polyploidy, mutagenesis and cell fusion techniques that do not use recombinant nucleic acids or a genetically modified organism in the process. Cloning and stem cell research, although not considered genetic engineering, are closely related and genetic engineering can be used within them. Synthetic biology is an emerging discipline that takes genetic engineering a step further by introducing artificially synthesized genetic material from raw materials into an organism.

If genetic material from another species is added to the host, the resulting organism is called transgenic. If genetic material from the same species or a species that can naturally breed with the host is used the resulting organism is called cisgenic. Genetic engineering can also be used to remove genetic material from the target organism, creating a knock out organism. In Europe genetic modification is synonymous with genetic engineering while within the United States of America it can also refer to conventional breeding methods.

History

Humans have altered the genomes of species for thousands of years through artificial selection and more recently mutagenesis. Genetic engineering as the direct manipulation of DNA by humans outside breeding and mutations has only existed since the 1970s. The term "genetic engineering" was first coined by Jack Williamson in his science fiction novel *Dragon's Island*, published in 1951, one year before DNA's role in heredity was confirmed by Alfred Hershey and Martha Chase, and two years before James Watson and Francis Crick showed that the DNA molecule has a double-helix structure.

In 1972 Paul Berg created the first recombinant DNA molecules by combined DNA from the monkey virus SV40 with that of the lambda virus. In 1973 Herbert Boyer and Stanley Cohen created the first transgenic organism by inserting antibiotic resistance genes into the plasmid of an *E. coli* bacterium. A year later Rudolf Jaenisch created a transgenic mouse by introducing foreign DNA into its embryo, making it the world's first transgenic animal. In 1976 Genentech, the first genetic engineering company was founded by Herbert Boyer and Robert Swanson and a year later and the company produced a human protein (somatostatin) in *E.coli*. Genentech announced the production of genetically engineered human insulin in 1978. In 1980, the U.S. Supreme Court in the Diamond v. Chakrabarty case ruled that genetically altered life could be patented. The insulin produced by bacteria, branded humulin, was approved for release by the Food and Drug Administration in 1982.

The first field trials of genetically engineered plants occurred in France and the USA in 1986, tobacco plants were engineered to be resistant to herbicides. The People's Republic of China was the first country to commercialize transgenic plants, introducing a virus-resistant tobacco in 1992. In 1994 Calgene attained approval to commercially release the Flavr Savr tomato, a tomato engineered to have a longer shelf life. In 1994, the European Union approved tobacco engineered to be resistant to the herbicide bromoxynil, making it the first genetically engineered crop commercialized in Europe. In 1995, Bt Potato was approved safe by the Environmental Protection Agency, making it

the first pesticide producing crop to be approved in the USA. In 2009 11 transgenic crops were grown commercially in 25 countries, the largest of which by area grown were the USA, Brazil, Argentina, India, Canada, China, Paraguay and South Africa.

In 2010, scientists at the J. Craig Venter Institute, announced that they had created the first synthetic bacterial genome, and added it to a cell containing no DNA. The resulting bacterium, named Synthia, was the world's first synthetic life form.

Process

Isolating the Gene

Elements of genetic engineering

First, the gene to be inserted into the genetically modified organism must be chosen and isolated. Presently, most genes transferred into plants provide protection against insects or tolerance to herbicides. In animals the majority of genes used are growth hormone genes. Once chosen the genes must be isolated. This typically involves multiplying the gene using polymerase chain reaction (PCR). If the chosen gene or the donor organism's genome has been well studied it may be present in a genetic library. If the DNA sequence is known, but no copies of the gene are available, it can be artificially synthesized. Once isolated, the gene is inserted into a bacterial plasmid.

Constructs

The gene to be inserted into the genetically modified organism must be combined with other genetic elements in order for it to work properly. The gene can also be modified at this stage for better expression or effectiveness. As well as the gene to be inserted most constructs contain a promoter and terminator region as well as a selectable marker gene. The promoter region initiates transcription of the gene and can be used to control the location and level of gene expression, while the terminator region ends transcription. The selectable marker, which in most cases confers antibiotic resistance to the organism it is expressed in, is needed to determine which cells are transformed with the new gene. The constructs are made using recombinant DNA techniques, such as restriction digests, ligations and molecular cloning.

Gene Targeting

The most common form of genetic engineering involves inserting new genetic material randomly within the host genome. Other techniques allow new genetic material to be inserted at a specific location in the host genome or generate mutations at desired genomic loci capable of knocking out endogenous genes. The technique of gene targeting uses homologous recombination to target desired changes to a specific endogenous gene. This tends to occur at a relatively low frequency in plants and animals and generally requires the use of selectable markers. The frequency of gene targeting can be greatly enhanced with the use of engineered nucleases such as zinc finger nucleases, engineered homing endonucleases, or nucleases created from TAL effectors. In addition to enhancing gene targeting, engineered nucleases can also be used to introduce mutations at endogenous genes that generate a gene knockout.

Transformation

A. tumefaciens attaching itself to a carrot cell

About 1% of bacteria are naturally able to take up foreign DNA but it can also be induced in other bacteria. Stressing the bacteria for example, with a heat shock or an electric shock, can make the cell membrane permeable to DNA that may then incorporate into their genome or exist as extrachromosomal DNA. DNA is generally inserted into animal cells using microinjection, where it can be injected through the cells nuclear envelope directly into the nucleus or through the use of viral vectors. In plants the DNA is generally inserted using *Agrobacterium*-mediated recombination or biolistics.

In *Agrobacterium*-mediated recombination the plasmid construct must also contain T-DNA. *Agrobacterium* naturally inserts DNA from a tumor inducing plasmid into any susceptible plant's genome it infects, causing crown gall disease. The T-DNA region of this plasmid is responsible for insertion of the DNA. The genes to be inserted are cloned into a binary vector, which contains T-DNA and can be grown in both *E. Coli* and *Agrobacterium*. Once the binary vector is constructed the plasmid is transformed into *Agrobacterium* containing no plasmids and plant cells are infected. The *Agrobacterium* will then naturally insert the genetic material into the plant cells.

In biolistics particles of gold or tungsten are coated with DNA and then shot into young plant cells or plant embryos. Some genetic material will enter the cells and transform them. This method can be used on plants that are not susceptible to *Agrobacterium* infection and also allows transformation of plant plastids. Another transformation method for plant and animal cells is electroporation. Electroporation involves subjecting the plant or animal cell to an electric shock, which can make the cell membrane permeable to plasmid DNA. In some cases the electroporated cells will incorporate the DNA into their genome. Due to the damage caused to the cells and DNA the transformation efficiency of biolistics and electroporation is lower than agrobacterial mediated transformation and microinjection.

Selection

Not all the organism's cells will be transformed with the new genetic material; in most cases a selectable marker is used to differentiate transformed from untransformed cells. If a cell has been successfully transformed with the DNA it will also contain the marker gene. By growing the cells in the presence of an antibiotic or chemical that selects or marks the cells expressing that gene it is possible to separate the transgenic events from the non-transgenic. Another method of screening involves using a DNA probe that will only stick to the inserted gene. A number of strategies have been developed that can remove the selectable marker from the mature transgenic plant.

Regeneration

As often only a single cell is transformed with genetic material the organism must be regrown from that single cell. As bacteria consist of a single cell and reproduce clonally regeneration is not necessary. In plants this is accomplished through the use of tissue culture. Each plant species has different requirements for successful regeneration through tissue culture. If successful an adult plant is produced that contains the transgene in every cell. In animals it is necessary to ensure that the inserted DNA is present in the embryonic stem cells. When the offspring is produced they can be screened for the presence of the gene. All offspring from the first generation will be heterozygous for the inserted gene and must be mated together to produce a homozygous animal.

Confirmation

Further tests using PCR, Southern Blots and Bioassays are needed to confirm that the gene is expressed and functions correctly. The organism's offspring are also tested to ensure that the trait can be inherited and that it follows a Mendelian inheritance pattern.

Applications

Genetic engineering has applications in medicine, research, industry and agriculture and can be used on a wide range of plants, animals and micro organism.

Medicine

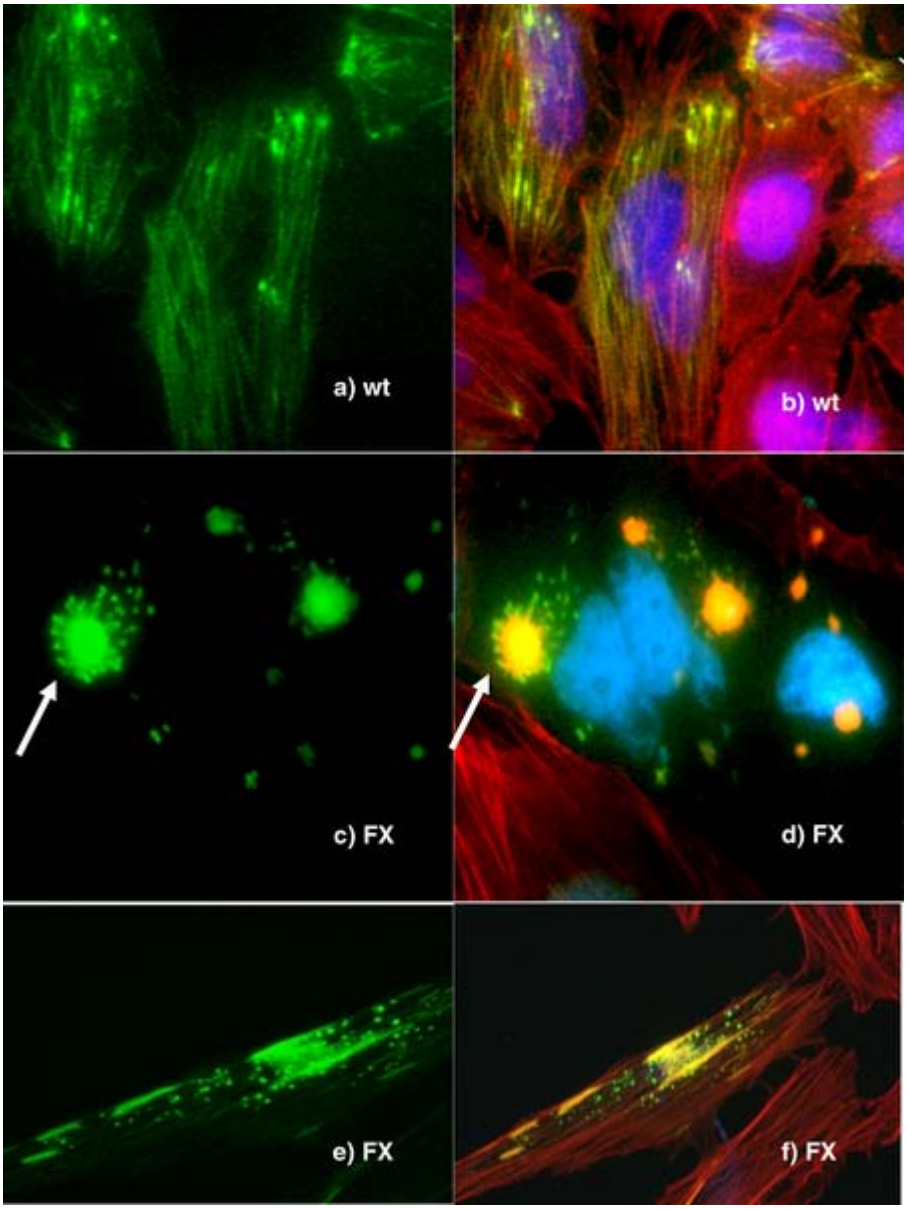
In medicine genetic engineering has been used to mass-produce insulin, human growth hormones, follistim (for treating infertility), human albumin, monoclonal antibodies, antihemophilic factors, vaccines and many other drugs. Vaccination generally involves injecting weak live, killed or inactivated forms of viruses or their toxins into the person being immunized. Genetically engineered viruses are being developed that can still confer immunity, but lack the infectious sequences. Mouse hybridomas, cells fused together to create monoclonal antibodies, have been humanised through genetic engineering to create human monoclonal antibodies.

Genetic engineering is used to create animal models of human diseases. Genetically modified mice are the most common genetically engineered animal model. They have been used to study and model cancer (the oncomouse), obesity, heart disease, diabetes, arthritis, substance abuse, anxiety, aging and Parkinson disease. Potential cures can be tested against these mouse models. Also genetically modified pigs have been bred with the aim of increasing the success of pig to human organ transplantation.

Gene therapy is the genetic engineering of humans by replacing defective human genes with functional copies. This can occur in somatic tissue or germline tissue. If the gene is inserted into the germline tissue it can be passed down to that person's descendants. Gene therapy has been used to treat patients suffering from immune deficiencies (notably Severe combined immunodeficiency) and trials have been carried out on other genetic disorders. The success of gene therapy so far has been limited and a patient (Jesse Gelsinger) has died during a clinical trial testing a new treatment. There are also ethical concerns should the technology be used not just for treatment, but for enhancement, modification or alteration of a human beings' appearance, adaptability, intelligence, character or behavior. The distinction between cure and enhancement can also be difficult to establish. Transhumanists consider the enhancement of humans desirable.

Research

Knockout mice



Human cells in which some proteins are fused with green fluorescent protein to allow them to be visualised

Genetic engineering is an important tool for natural scientists. Genes and other genetic information from a wide range of organisms are transformed into bacteria for storage and modification, creating genetically modified bacteria in the process. Bacteria are cheap, easy to grow, clonal, multiply quickly, relatively easy to transform and can be stored at -80°C almost indefinitely. Once a gene is isolated it can be stored inside the bacteria providing an unlimited supply for research.

Organisms are genetically engineered to discover the functions of certain genes. This could be the effect on the phenotype of the organism, where the gene is expressed or what other genes it interacts with. These experiments generally involve loss of function, gain of function, tracking and expression.

- **Loss of function experiments**, such as in a gene knockout experiment, in which an organism is engineered to lack the activity of one or more genes. A knockout experiment involves the creation and manipulation of a DNA construct *in vitro*, which, in a simple knockout, consists of a copy of the desired gene, which has been altered such that it is non-functional. Embryonic stem cells incorporate the altered gene, which replaces the already present functional copy. These stem cells are injected into blastocysts, which are implanted into surrogate mothers. This allows the experimenter to analyze the defects caused by this mutation and thereby determine the role of particular genes. It is used especially frequently in developmental biology. Another method, useful in organisms such as Drosophila (fruit fly), is to induce mutations in a large population and then screen the progeny for the desired mutation. A similar process can be used in both plants and prokaryotes.
- **Gain of function experiments**, the logical counterpart of knockouts. These are sometimes performed in conjunction with knockout experiments to more finely establish the function of the desired gene. The process is much the same as that in knockout engineering, except that the construct is designed to increase the function of the gene, usually by providing extra copies of the gene or inducing synthesis of the protein more frequently.
- **Tracking experiments**, which seek to gain information about the localization and interaction of the desired protein. One way to do this is to replace the wild-type gene with a 'fusion' gene, which is a juxtaposition of the wild-type gene with a reporting element such as green fluorescent protein (GFP) that will allow easy visualization of the products of the genetic modification. While this is a useful technique, the manipulation can destroy the function of the gene, creating secondary effects and possibly calling into question the results of the experiment. More sophisticated techniques are now in development that can track protein products without mitigating their function, such as the addition of small sequences that will serve as binding motifs to monoclonal antibodies.
- **Expression studies** aim to discover where and when specific proteins are produced. In these experiments, the DNA sequence before the DNA that codes for a protein, known as a gene's promoter, is reintroduced into an organism with the protein coding region replaced by a reporter gene such as GFP or an enzyme that catalyzes the production of a dye. Thus the time and place where a particular protein is produced can be observed. Expression studies can be taken a step further by altering the promoter to find which pieces are crucial for the proper expression of the gene and are actually bound by transcription factor proteins; this process is known as promoter bashing.

Industrial

By engineering genes into bacterial plasmids it is possible to create a biological factory that can produce proteins and enzymes. Some genes do not work well in bacteria, so yeast, a eukaryote, can also be used. Bacteria and yeast factories have been used to produce medicines such as insulin, human growth hormone, and vaccines, supplements such as tryptophan, aid in the production of food (chymosin in cheese making) and fuels.

Other applications involving genetically engineered bacteria being investigated involve making the bacteria perform tasks outside their natural cycle, such as cleaning up oil spills, carbon and other toxic waste.

Agriculture

Bt-toxins present in peanut leaves (bottom image) protect it from extensive damage caused by European corn borer larvae (top image).

One of the best-known and controversial applications of genetic engineering is the creation of genetically modified food. There are three generations of genetically modified crops. First generation crops have been commercialized and most provide protection from insects and/or resistance to herbicides. There are also fungal and virus resistant crops developed or in development. They have been developed to make the insect and weed management of crops easier and can indirectly increase crop yield.

The second generation of genetically modified crops being developed aim to directly improve yield by improving salt, cold or drought tolerance and to increase the nutritional value of the crops. The third generation consists of pharmaceutical crops, crops that contain edible vaccines and other drugs. Some agriculturally important animals have been genetically modified with growth hormones to increase their size while others have been engineered to express drugs and other proteins in their milk.

The genetic engineering of agricultural crops can increase the growth rates and resistance to different diseases caused by pathogens and parasites. This is beneficial as it can greatly increase the production of food sources with the usage of fewer resources that would be required to host the world's growing populations. These modified crops would also reduce the usage of chemicals, such as fertilizers and pesticides, and therefore decrease the severity and frequency of the damages produced by these chemical pollution.

Ethical and safety concerns have been raised around the use of genetically modified food. A major safety concern relates to the human health implications of eating genetically modified food, in particular whether toxic or allergic reactions could occur. Gene flow into related non-transgenic crops, off target effects on beneficial organisms and the impact on biodiversity are important environmental issues. Ethical concerns involve religious issues, corporate control of the food supply, intellectual property rights and the level of labeling needed on genetically modified products.

Other uses

In materials science, a genetically modified virus has been used to construct a more environmentally friendly lithium-ion battery. Some bacteria have been genetically engineered to create black and white photographs while others have potential to be used as sensors by expressing a fluorescent protein under certain environmental conditions. Genetic engineering is also being used to create BioArt and novelty items such as blue roses, and glowing fish.

Opposition and criticism

A 2010 study of Canola found transgenes in 80% of wild (uncultivated or "feral") varieties in North Dakota, meaning 80% of the plants which had established themselves in the area were genetically engineered varieties. The researchers stated that "we found the highest densities of [such transgene-containing] plants near agricultural fields and along major freeways, but we were also finding plants in the middle of nowhere" adding that "over time,..the build-up of different types of herbicide resistance in feral [natural] canola and closely related weeds, like field mustard, could make it more difficult to manage these plants using herbicides."

Chapter- 4

Human Genetic Engineering

Human genetic engineering is the alteration of an individual's genotype with the aim of choosing the phenotype of a newborn or changing the existing phenotype of a child or adult. It holds the promise of curing genetic diseases like cystic fibrosis, and increasing the immunity of people to viruses. It is speculated that genetic engineering could be used to change physical appearance, metabolism, and even improve mental faculties like memory and intelligence, although for now these uses seem to be of lower priority to researchers and are therefore limited to science fiction.

History

The first gene therapy trials on humans began in 1990 on patients with Severe Combined Immunodeficiency (SCID). In 2000, the first gene therapy "success" resulted in SCID patients with a functional immune system. These trials were stopped when it was discovered that two of ten patients in one trial had developed leukemia resulting from the insertion of the gene-carrying retrovirus near an oncogene. In 2007, four of the ten patients had developed leukemia . Work is now focusing on correcting the gene without triggering an oncogene.

Trial treatments of SCID have been gene therapy's only success; since 1999, gene therapy has restored the immune systems of at least 17 children with two forms (ADA-SCID and X-SCID) of the disorder.

Human genetic engineering is already being used on a small scale to allow infertile women with genetic defects in their mitochondria to have children. Healthy human eggs from a second mother are used. The child produced this way has genetic information from two mothers and one father. The changes made are germline changes and will likely be passed down from generation to generation, and, thus, are a permanent change to the human genome.

Other forms of human genetic engineering are still theoretical. Recombinant DNA research is usually performed to study gene expression and various human diseases. Some drastic demonstrations of gene modification have been made with mice and other animals, however, testing on humans is generally considered off-limits. In some instances

changes are usually brought about by removing genetic material from one organism and transferring them into another species.

Methods

Somatic

Somatic genetic engineering involves adding genes to cells other than egg or sperm cells. For example, if a person had a disease caused by a defective gene, a healthy gene could be added to the affected cells to treat the disorder. As of now, this is likely to take the form of gene therapy. The distinguishing characteristic of somatic engineering is that it is non-inheritable, i.e. the new gene would not be passed to the recipient's offspring.

There are two techniques researchers are currently experimenting with:

- Viruses are good at injecting their DNA payload into human cells and reproducing it. By adding the desired DNA to the DNA of non-pathogenic virus, a small amount of virus will reproduce the desired DNA and spread it all over the body.
- Manufacture large quantities of DNA, and somehow package it to induce the target cells to accept it, either as an addition to one of the original 23 chromosomes, or as an independent 24th human artificial chromosome.

Germline

Germline engineering involves changing genes in eggs, sperm, or very early embryos. This type of engineering is inheritable, meaning that the modified genes would appear not only in offspring that resulted from the procedure, but also in subsequent generations.

Uses

Two motivators of human genetic engineering are referred to as "negative" and "positive". The former aims to remove genetic disorders and the latter aims to alter phenotypic expression to result in an enhanced being.

Negative genetic engineering (cures and treatments)

When treating problems that arise from genetic disorder, one solution is gene therapy, also known as negative genetic engineering. A genetic disorder is a condition caused by the genetic code of the individual, such as spina bifida or autism. When this happens, genes may be expressed in unfavorable ways or not at all, and this generally leads to further complications.

The idea of gene therapy is that a non-pathogenic virus or other delivery systems can be used to insert into DNA—a good copy of the gene—into cells of the living individual. The modified cells would divide as normal and each division would produce cells that

express the desired trait. The result would be that he/she would then have the ability to express the trait that was previously absent, at least partially. This form of genetic engineering could help alleviate many problems, such as diabetes, cystic fibrosis, or other genetic diseases.

Positive genetic engineering (enhancement)

The potential of genetic engineering to cure medical conditions opens the question of exactly what such a condition is. Some view aging and death as medical conditions and therefore potential targets for engineering solutions. They see human genetic engineering potentially as a key tool in this. The difference between cure and enhancement from this perspective is merely one of degree. Theoretically genetic engineering could be used to drastically change people's genomes, which could enable people to regrow limbs and other organs, perhaps even extremely complex ones such as the spine.

It could also be used to make people smarter, stronger, or to increase the capacity of the lungs, among other things. If a gene exists in nature, perhaps it could be changed into a human cell. In this view, there is no qualitative difference (only a quantitative one) between, for instance, a genetic intervention to cure muscular atrophy, and a genetic intervention to improve muscle function even when those muscles are functioning at or below the human average (since there is also an average muscle function for those with a particular type of dystrophy, which the treatment would improve upon).

Others feel, there is an important distinction between using genetic technologies to treat those who are suffering, and to make those who are already healthy seem more superior to the average person. Though theory and speculation suggest, that genetic engineering could be used to make people stronger, faster, smarter, or to increase lung capacity. The AAAS report finds that there is little evidence to support this theory. Can this currently be done without very unsafe and therefore unethical human experiments. Because different cells have different tasks, changing one cell to do a function differently, will not only affect that one task, but it can affect many other tasks as well.

Controversy

Ethics

The genetic engineering of humans has raised many controversial ethical issues. While negative genetic engineering (gene therapy) does indeed raise a debate, the use of genetic engineering for human enhancement arouses the strongest feelings on both sides.

Genetic engineering is tested on animals, often including primates. Some animal rights activists find this inhumane.

Genetic engineering must be used to cure peoples with diabetes. It is possible to extract genes from cells which are called beta cells and then to insert the insulin producing genes into a bacterium. Then the bacterium will start producing insulin. Genetic modification of

embryos can pose an ethical question about the rights of the baby. One belief is that every fetus should be free to not be genetically modified. Others believe that parents hold the rights to change their unborn children. Still others believe that every child should have the right to be born free from preventable diseases.

Molecular Biologist Lee M. Silver believes that unlike Aldous Huxley's Brave New World, where a totalitarian government controls all of the genetic enhancements (they actually use eugenics instead of direct genetic modification) in society, the use of gene therapy to design children will be spread through what he calls "free market eugenics" (Silver 315). Wealthy families will opt to design their child with genetic advantages because other families are doing so, and everybody wants to provide their newborn child with the best opportunities in life, with a leg up on the competition.

The greatest fear for Silver is that we will design so many children with germline gene therapy, that the families wealthy enough to design their children, will pass down these enhanced traits to future generations. This gene therapy will obviously cost money, and the less wealthy families will be left to procreate naturally, and introduce their children into the world disadvantaged from their first breath.

The impact on society will be a new alignment of classes, no longer will we separate people by their ethnic differences, the new division will be between what Silver calls 'the naturals' and 'the GenRich', or genetically enhanced. The major worry here is that the 'genetic gulf' between these two classes will become so wide that humans will become separate species (Silver 313).

In popular culture

- Maximum Ride series by James Patterson: The main characters are six human children who had bird DNA injected into them while they were in their mothers' wombs.

- Mobile Suit Gundam SEED (anime): Set in a world in which genetically modified humans, termed 'Coordinators', have been ostracized and isolated from unmodified humans, termed 'Naturals'. Due to extreme differences in mental and physical abilities between the two groups, racial, economic, and political issues have arisen, culminating in war. Gundam Seed addresses such concerns as animosity caused by the jealousy of Naturals over Coordinator abilities, both groups looking down on one another as being lesser life forms, and genocidal factions emerging on both sides. The series primarily explores these issues from the point of view of a Coordinator protagonist who finds himself fighting on the side of the Naturals, as his childhood friend has become a member of the Coordinator military, giving a perspective on both sides of the conflict.

- *Gattaca* (film): Presents a biopunk vision of a society driven by new eugenics. Children of the middle and upper classes are selected through preimplantation genetic diagnosis to ensure they possess the best hereditary traits of their parents.

- Oryx and Crake by Margaret Atwood: Apocalyptic pseudo-dystopian story, one of the chief plot points of which involves the genetic engineering of a new type of transhuman and the pathological decimation of *Homo sapiens*.

- Bioshock (Video Game): The main enemies the player encounters over the course of the game are known as splicers, so called for their genetic manipulation, or gene splicing. In the game a compound called ADAM is responsible for the genetic manipulation. The compound is harvested from a species of sea slugs, it acts like a seemingly benign form of cancer, destroying native cells and replacing them with the unstable stem versions. The splicers have all become so addicted to the ADAM that they will kill anything or anyone for it.

- Old Man's War series by John Scalzi: To create an army of soldiers capable of defending the human race from endless alien hordes the Colonial Defense Forces recruits 75 year olds and gives them new younger bodies capable of super human feats.

- Batman Beyond: In the episode *Splicers* and making appearances later in the series, a new fad of splicing animal genes for cosmetic and enhancement purposes makes the teen scene. When research shows that splicing increases aggression in users, resulting it being banned in Gotham, only finding a place in the criminal underworld later. Notable Splicers are Woof of the Jokerz (spliced with hyena DNA), and the Zander (spliced with T-Rex DNA), leader of KOBRA.

- Halo (series) (Video Game): the protagonist and several other characters in the Halo universe known as Spartans have all gone through comprehensive genetic augmentation, making them almost super human. The augmentations vary from Muscular Enhancement Injections to a Catalytic Thyroid Implant. The effects include nearly unbreakable bones, increased reflexes and increased muscle tissue with increased density. The SPARTAN-III program also included a mutagen that increased aggression and injury tolerance.

- Deus Ex: The protagonists of both games in the series are genetically modified by injections called nano-augs (Deus Ex) and bio mods (Invisible War). These nanite injections alter the host's genes to enhance them with new skill that would help them through different obstacles in the game. Both the protagonists and antagonists are bio-modified along with other support characters and neutral characters.

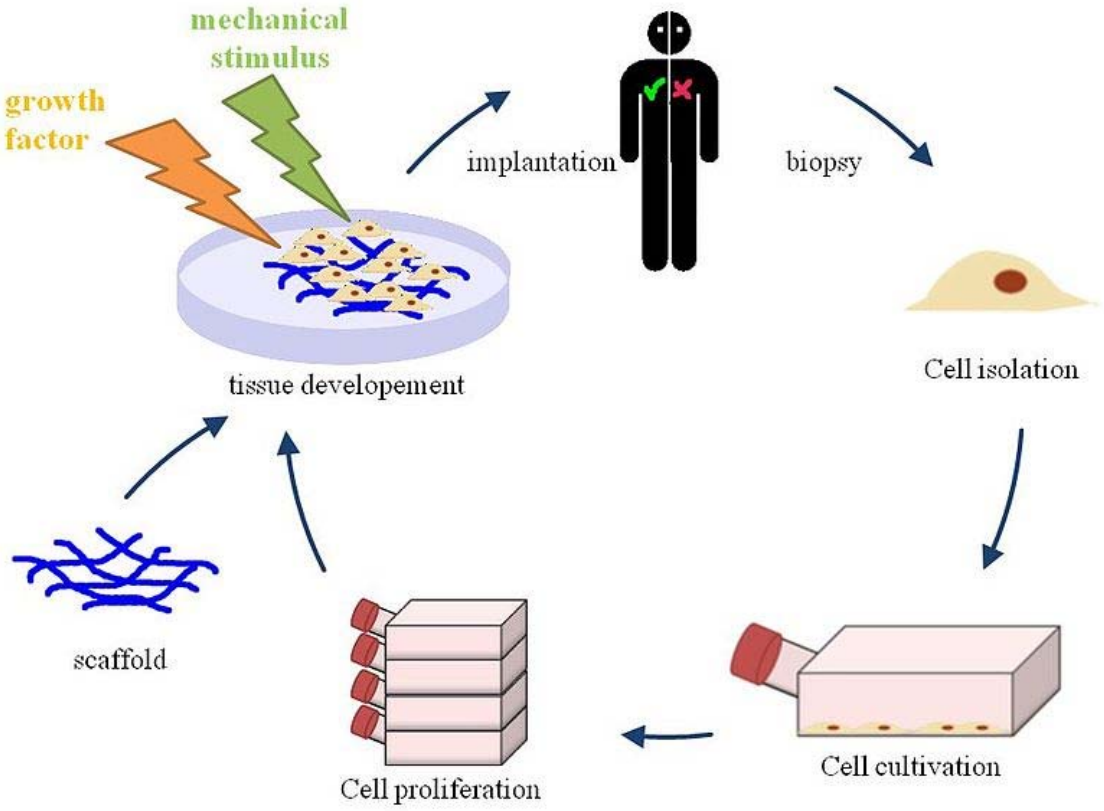
- PROTOTYPE: The story is centered around a genetic engineering company called Gentek. The engineers in Gentek modified a chimeran-virus to make it 10 times as deadly, to 'unlock' previously dormant parts of a subjects DNA, and they made it to the point of where it could copy those infected with it down to the genetic level. The new 'infected' being then has complete and total control over its genetic structure, allowing it to develop instantaneous shape-shifting abilities. However, the game reveals that the only 'infected' being that can do this is a

person already dead (or dying) when the virus enters the bloodstream. The virus must also be in a state of emergency to survive and not die out.

- Warhammer 40,000: In the games, and table-top series, Space marines are the result of Genetic Engineering, by replacing organs, and limbs that have been engineered. The Marine himself, also goes through rigorous physical and mental training, to make him accustom to the newly added appendages and organs.

Chapter- 5

Tissue Engineering

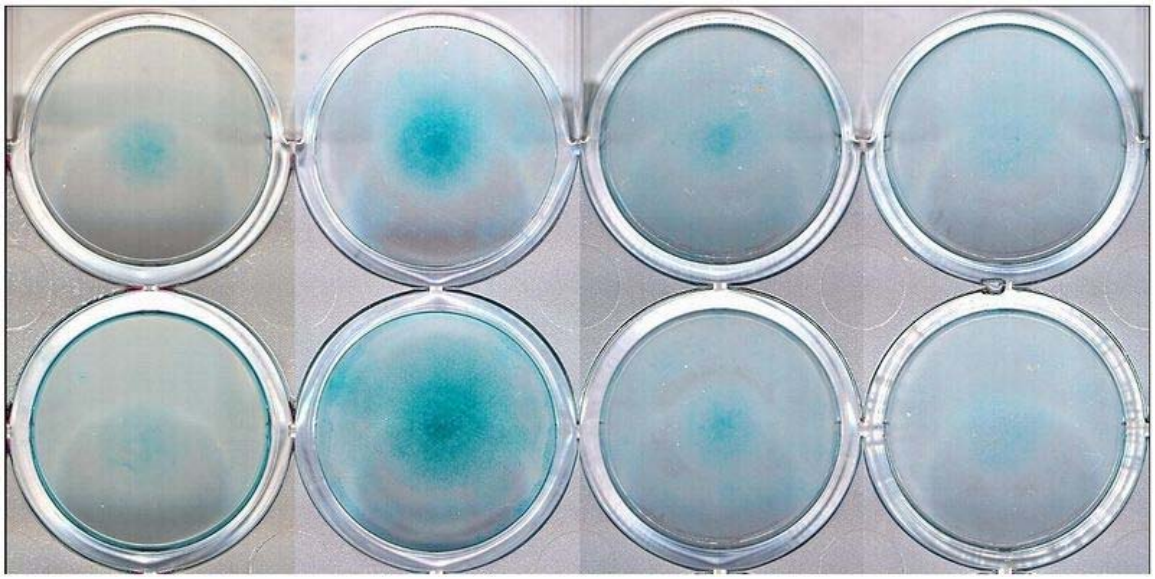


Principle of tissue engineering

Tissue engineering was once categorized as a sub-field of bio materials, but having grown in scope and importance it can be considered as a field in its own right. It is the use of a combination of cells, engineering and materials methods, and suitable biochemical and physio-chemical factors to improve or replace biological functions. While most definitions of tissue engineering cover a broad range of applications, in practice the term is closely associated with applications that repair or replace portions of or whole tissues (i.e., bone, cartilage, blood vessels, bladder, skin etc.). Often, the tissues involved require certain mechanical and structural properties for proper functioning. The term has also been applied to efforts to perform specific biochemical functions using cells within an artificially-created support system (e.g. an artificial pancreas, or a bio artificial

liver). The term **regenerative medicine** is often used synonymously with tissue engineering, although those involved in regenerative medicine place more emphasis on the use of stem cells to produce tissues.

Overview



Micro-mass cultures of C3H-10T1/2 cells at varied oxygen tensions stained with Alcian blue.

A commonly applied definition of tissue engineering, as stated by Langer and Vacanti, is "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ". Tissue engineering has also been defined as "understanding the principles of tissue growth, and applying this to produce functional replacement tissue for clinical use." A further description goes on to say that an "underlying supposition of tissue engineering is that the employment of natural biology of the system will allow for greater success in developing therapeutic strategies aimed at the replacement, repair, maintenance, and/or enhancement of tissue function."

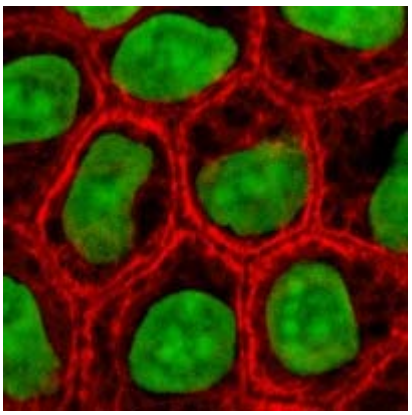
Powerful developments in the multidisciplinary field of tissue engineering have yielded a novel set of tissue replacement parts and implementation strategies. Scientific advances in biomaterials, stem cells, growth and differentiation factors, and biomimetic environments have created unique opportunities to fabricate tissues in the laboratory from combinations of engineered extracellular matrices ("scaffolds"), cells, and biologically active molecules. Among the major challenges now facing tissue engineering is the need for more complex functionality, as well as both functional and biomechanical stability in laboratory-grown tissues destined for transplantation. The continued success of tissue engineering, and the eventual development of true human replacement parts, will grow from the convergence of engineering and basic research advances in tissue, matrix, growth factor, stem cell, and developmental biology, as well as materials science and bio informatics.

In 2003, the NSF published a report entitled "The Emergence of Tissue Engineering as a Research Field" , which gives a thorough description of the history of this field.

Examples

- Tissue engineered autologous heartvalves and vessels - workgroup of Dr.med.S.Jockenhoevel at the Department of Applied Medical Engineering (RWTH-Aachen University,Germany)
- In vitro meat — Edible artificial animal muscle tissue cultured *in vitro*.
- Bioartificial liver device — several research efforts have produced hepatic assist devices utilizing living hepatocytes.
- Artificial pancreas — research involves using islet cells to produce and regulate insulin, particularly in cases of diabetes.
- Artificial bladders — Anthony Atala (Wake Forest University) has successfully implanted artificially grown bladders into seven out of approximately 20 human test subjects as part of a long-term experiment.
- Cartilage — lab-grown tissue was successfully used to repair knee cartilage.
- Doris Taylor's heart in a jar
- Tissue-engineered airway
- Artificial skin constructed from human skin cells embedded in collagen
- Artificial bone marrow
- Artificial bone
- Artificial penis

Cells as building blocks



Stained cells in culture

Tissue engineering utilizes living cells as engineering materials. Examples include using living fibroblasts in skin replacement or repair, cartilage repaired with living chondrocytes, or other types of cells used in other ways.

Cells became available as engineering materials when scientists at Geron Corp. discovered how to extend telomeres in 1998, producing immortalized cell lines. Before this, laboratory cultures of healthy, noncancerous mammalian cells would only divide a fixed number of times, up to the Hayflick limit.

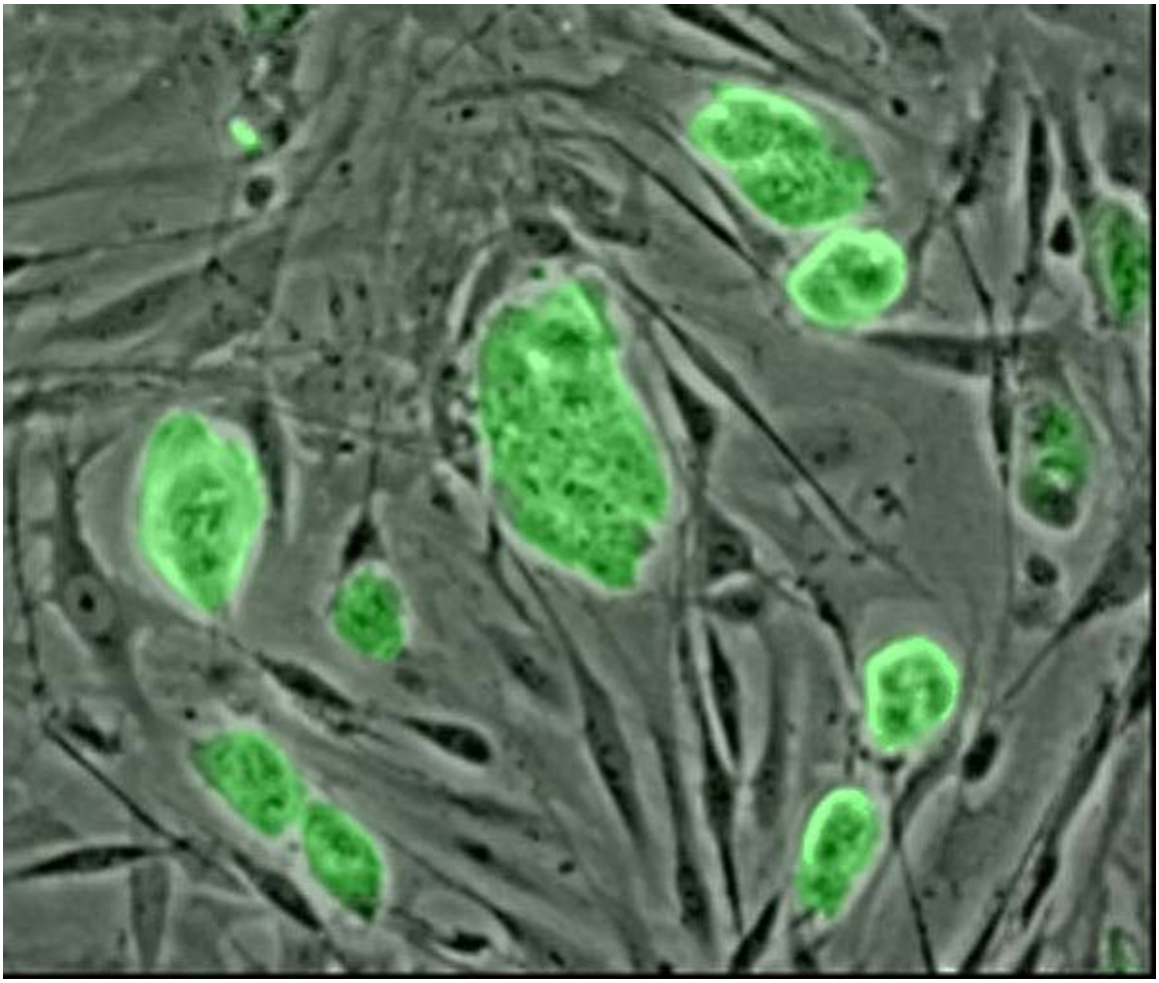
Extraction

From fluid tissues such as blood, cells are extracted by bulk methods, usually centrifugation or apheresis. From solid tissues, extraction is more difficult. Usually the tissue is minced, and then digested with the enzymes trypsin or collagenase to remove the extracellular matrix that holds the cells. After that, the cells are free floating, and extracted using centrifugation or apheresis.
Digestion with trypsin is very dependent on temperature. Higher temperatures digest the matrix faster, but create more damage. Collagenase is less temperature dependent, and damages fewer cells, but takes longer and is a more expensive reagent.

Types of cells

Cells are often categorized by their source:

- **Autologous** cells are obtained from the same individual to which they will be reimplanted. Autologous cells have the fewest problems with rejection and pathogen transmission, however in some cases might not be available. For example in genetic disease suitable autologous cells are not available. Also very ill or elderly persons, as well as patients suffering from severe burns, may not have sufficient quantities of autologous cells to establish useful cell lines. Moreover since this category of cells needs to be harvested from the patient, there are also some concerns related to the necessity of performing such surgical operations that might lead to donor site infection or chronic pain. Autologous cells also must be cultured from samples before they can be used: this takes time, so autologous solutions may not be very quick. Recently there has been a trend towards the use of mesenchymal stem cells from bone marrow and fat. These cells can differentiate into a variety of tissue types, including bone, cartilage, fat, and nerve. A large number of cells can be easily and quickly isolated from fat, thus opening the potential for large numbers of cells to be quickly and easily obtained.



Mouse embryonic stem cells. **More lab photos**

- **Allogeneic** cells come from the body of a donor of the same species. While there are some ethical constraints to the use of human cells for *in vitro* studies, the employment of dermal fibroblasts from human foreskin has been demonstrated to be immunologically safe and thus a viable choice for tissue engineering of skin.

- **Xenogenic** cells are these isolated from individuals of another species. In particular animal cells have been used quite extensively in experiments aimed at the construction of cardiovascular implants.

- **Syngenic** or **isogenic** cells are isolated from genetically identical organisms, such as twins, clones, or highly inbred research animal models.

- **Primary** cells are from an organism.

- **Secondary** cells are from a cell bank.

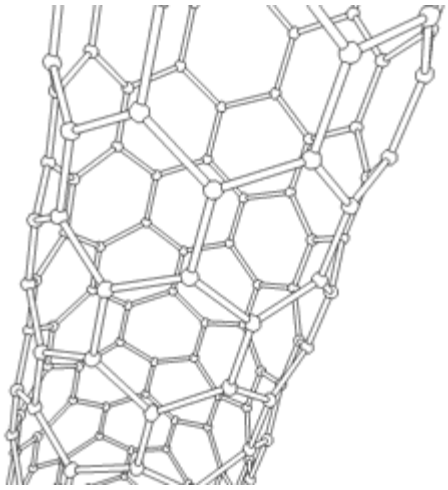
- **Stem cells** are undifferentiated cells with the ability to divide in culture and give rise to different forms of specialized cells. According to their source stem cells are

divided into "adult" and "embryonic" stem cells, the first class being multipotent and the latter mostly pluripotent; some cells are totipotent, in the earliest stages of the embryo. While there is still a large ethical debate related with the use of embryonic stem cells, it is thought that stem cells may be useful for the repair of diseased or damaged tissues, or may be used to grow new organs.

Scaffolds

Cells are often implanted or 'seeded' into an artificial structure capable of supporting three-dimensional tissue formation. These structures, typically called scaffolds, are often critical, both *ex vivo* as well as *in vivo*, to recapitulating the *in vivo* milieu and allowing cells to influence their own microenvironments. Scaffolds usually serve at least one of the following purposes:

- Allow cell attachment and migration
- Deliver and retain cells and biochemical factors
- Enable diffusion of vital cell nutrients and expressed products
- Exert certain mechanical and biological influences to modify the behaviour of the cell phase



This animation of a rotating Carbon nanotube shows its 3D structure. Carbon nanotubes are among the numerous candidates for tissue engineering scaffolds since they are biocompatible, resistant to biodegradation and can be functionalized with biomolecules. However, the possibility of toxicity with non-biodegradable nano-materials is not fully understood.

To achieve the goal of tissue reconstruction, scaffolds must meet some specific requirements. A high porosity and an adequate pore size are necessary to facilitate cell seeding and diffusion throughout the whole structure of both cells and nutrients. Biodegradability is often an essential factor since scaffolds should preferably be absorbed by the surrounding tissues without the necessity of a surgical removal. The rate at which degradation occurs has to coincide as much as possible with the rate of tissue formation: this means that while cells are fabricating their own natural matrix structure around

themselves, the scaffold is able to provide structural integrity within the body and eventually it will break down leaving the neotissue, newly formed tissue which will take over the mechanical load. Injectability is also important for clinical uses. Recent research on organ printing is showing how crucial a good control of the 3D environment is to insure reproducibility of experiments and offer better results.

Materials

Many different materials (natural and synthetic, biodegradable and permanent) have been investigated. Most of these materials have been known in the medical field before the advent of tissue engineering as a research topic, being already employed as bioresorbable sutures. Examples of these materials are collagen and some polyesters.

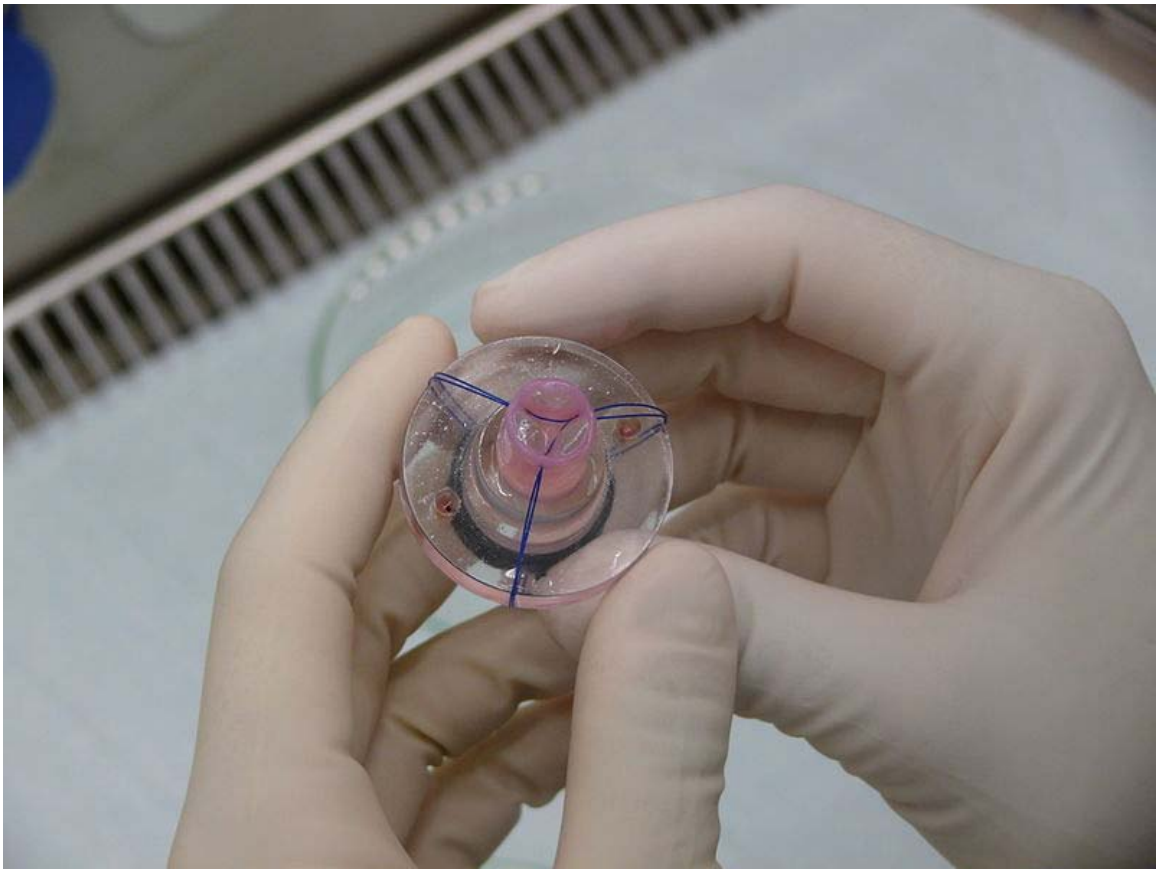
New biomaterials have been engineered to have ideal properties and functional customization: injectability, synthetic manufacture, biocompatibility, non-immunogenicity, transparency, nano-scale fibers, low concentration, resorption rates, etc. PuraMatrix, originating from the MIT labs of Zhang, Rich, Grodzinsky and Langer is one of these new biomimetic scaffold families which has now been commercialized and is impacting clinical tissue engineering.

A commonly used synthetic material is PLA - polylactic acid. This is a polyester which degrades within the human body to form lactic acid, a naturally occurring chemical which is easily removed from the body. Similar materials are polyglycolic acid (PGA) and polycaprolactone (PCL): their degradation mechanism is similar to that of PLA, but they exhibit respectively a faster and a slower rate of degradation compared to PLA.

Scaffolds may also be constructed from natural materials: in particular different derivatives of the extracellular matrix have been studied to evaluate their ability to support cell growth. Proteic materials, such as collagen or fibrin, and polysaccharidic materials, like chitosan or glycosaminoglycans (GAGs), have all proved suitable in terms of cell compatibility, but some issues with potential immunogenicity still remains. Among GAGs hyaluronic acid, possibly in combination with cross linking agents (e.g. glutaraldehyde, water soluble carbodiimide, etc...), is one of the possible choices as scaffold material. Functionalized groups of scaffolds may be useful in the delivery of small molecules (drugs) to specific tissues. Another form of scaffold under investigation is decellularised tissue extracts whereby the remaining cellular remnants/extracellular matrices act as the scaffold.

Synthesis

Tissue engineered vascular graft



Tissue engineered heart valve

A number of different methods have been described in literature for preparing porous structures to be employed as tissue engineering scaffolds. Each of these techniques presents its own advantages, but none are free of drawbacks.

- **Nanofiber Self-Assembly**: Molecular self-assembly is one of the few methods for creating biomaterials with properties similar in scale and chemistry to that of the natural in vivo extracellular matrix (ECM). Moreover, these hydrogel scaffolds have shown superiority in in vivo toxicology and biocompatibility compared to traditional macroscaffolds and animal-derived materials.

- **Textile technologies**: These techniques include all the approaches that have been successfully employed for the preparation of non-woven meshes of different polymers. In particular, non-woven polyglycolide structures have been tested for tissue engineering applications: such fibrous structures have been found useful to grow different types of cells. The principal drawbacks are related to the difficulties in obtaining high porosity and regular pore size.

- **Solvent Casting & Particulate Leaching (SCPL)**: This approach allows for the preparation of porous structures with regular porosity, but with a limited thickness. First, the polymer is dissolved into a suitable organic solvent (e.g. polylactic acid could be dissolved into dichloromethane), then the solution is cast

into a mold filled with porogen particles. Such porogen can be an inorganic salt like sodium chloride, crystals of saccharose, gelatin spheres or paraffin spheres. The size of the porogen particles will affect the size of the scaffold pores, while the polymer to porogen ratio is directly correlated to the amount of porosity of the final structure. After the polymer solution has been cast the solvent is allowed to fully evaporate, then the composite structure in the mold is immersed in a bath of a liquid suitable for dissolving the porogen: water in the case of sodium chloride, saccharose and gelatin or an aliphatic solvent like hexane for use with paraffin. Once the porogen has been fully dissolved, a porous structure is obtained. Other than the small thickness range that can be obtained, another drawback of SCPL lies in its use of organic solvents which must be fully removed to avoid any possible damage to the cells seeded on the scaffold.

- **Gas Foaming**: To overcome the need to use organic solvents and solid porogens, a technique using gas as a porogen has been developed. First, disc-shaped structures made of the desired polymer are prepared by means of compression molding using a heated mold. The discs are then placed in a chamber where they are exposed to high pressure CO_2 for several days. The pressure inside the chamber is gradually restored to atmospheric levels. During this procedure the pores are formed by the carbon dioxide molecules that abandon the polymer, resulting in a sponge-like structure. The main problems resulting from such a technique are caused by the excessive heat used during compression molding (which prohibits the incorporation of any temperature labile material into the polymer matrix) and by the fact that the pores do not form an interconnected structure.

- **Emulsification/Freeze-drying**: This technique does not require the use of a solid porogen like SCPL. First, a synthetic polymer is dissolved into a suitable solvent (e.g. polylactic acid in dichloromethane) then water is added to the polymeric solution and the two liquids are mixed in order to obtain an emulsion. Before the two phases can separate, the emulsion is cast into a mold and quickly frozen by means of immersion into liquid nitrogen. The frozen emulsion is subsequently freeze-dried to remove the dispersed water and the solvent, thus leaving a solidified, porous polymeric structure. While emulsification and freeze-drying allow for a faster preparation when compared to SCPL (since it does not require a time consuming leaching step), it still requires the use of solvents. Moreover, pore size is relatively small and porosity is often irregular. Freeze-drying by itself is also a commonly employed technique for the fabrication of scaffolds. In particular, it is used to prepare collagen sponges: collagen is dissolved into acidic solutions of acetic acid or hydrochloric acid that are cast into a mold, frozen with liquid nitrogen and then lyophilized.

- **Thermally Induced Phase Separation (TIPS)**: Similar to the previous technique, this phase separation procedure requires the use of a solvent with a low melting point that is easy to sublime. For example dioxane could be used to dissolve polylactic acid, then phase separation is induced through the addition of a

small quantity of water: a polymer-rich and a polymer-poor phase are formed. Following cooling below the solvent melting point and some days of vacuum-drying to sublime the solvent, a porous scaffold is obtained. Liquid-liquid phase separation presents the same drawbacks of emulsification/freeze-drying.

- **Electrospinning**: A highly versatile technique that can be used to produce continuous fibers from submicron to nanometer diameters. In a typical electrospinning set-up, a solution is fed through a spinneret and a high voltage is applied to the tip. The buildup of electrostatic repulsion within the charged solution, causes it to eject a thin fibrous stream. A mounted collector plate or rod with an opposite or grounded charge draws in the continuous fibers, which arrive to form a highly porous network. The primary advantages of this technique are its simplicity and ease of variation. At a laboratory level, a typical electrospinning set-up only requires a high voltage power supply (up to 30 kV), a syringe, a flat tip needle and a conducting collector. By modifying variables such as the distance to collector, magnitude of applied voltage, or solution flow rate—researchers can dramatically change the overall scaffold architecture.

- **CAD/CAM Technologies**: Because most of the above techniques are limited when it comes to the control of porosity and pore size, computer assisted design and manufacturing techniques have been introduced to tissue engineering. First, a three-dimensional structure is designed using CAD software, then the scaffold is realized by using ink-jet printing of polymer powders or through Fused Deposition Modeling of a polymer melt.

Assembly methods

One of the continuing, persistent problems with tissue engineering is mass transport limitations. Engineered tissues generally lack an initial blood supply, thus making it difficult for any implanted cells to obtain sufficient oxygen and nutrients to survive, and/or function properly.

Self-assembly may play an important role here, both from the perspective of encapsulating cells and proteins, as well as creating scaffolds on the right physical scale for engineered tissue constructs and cellular ingrowth.

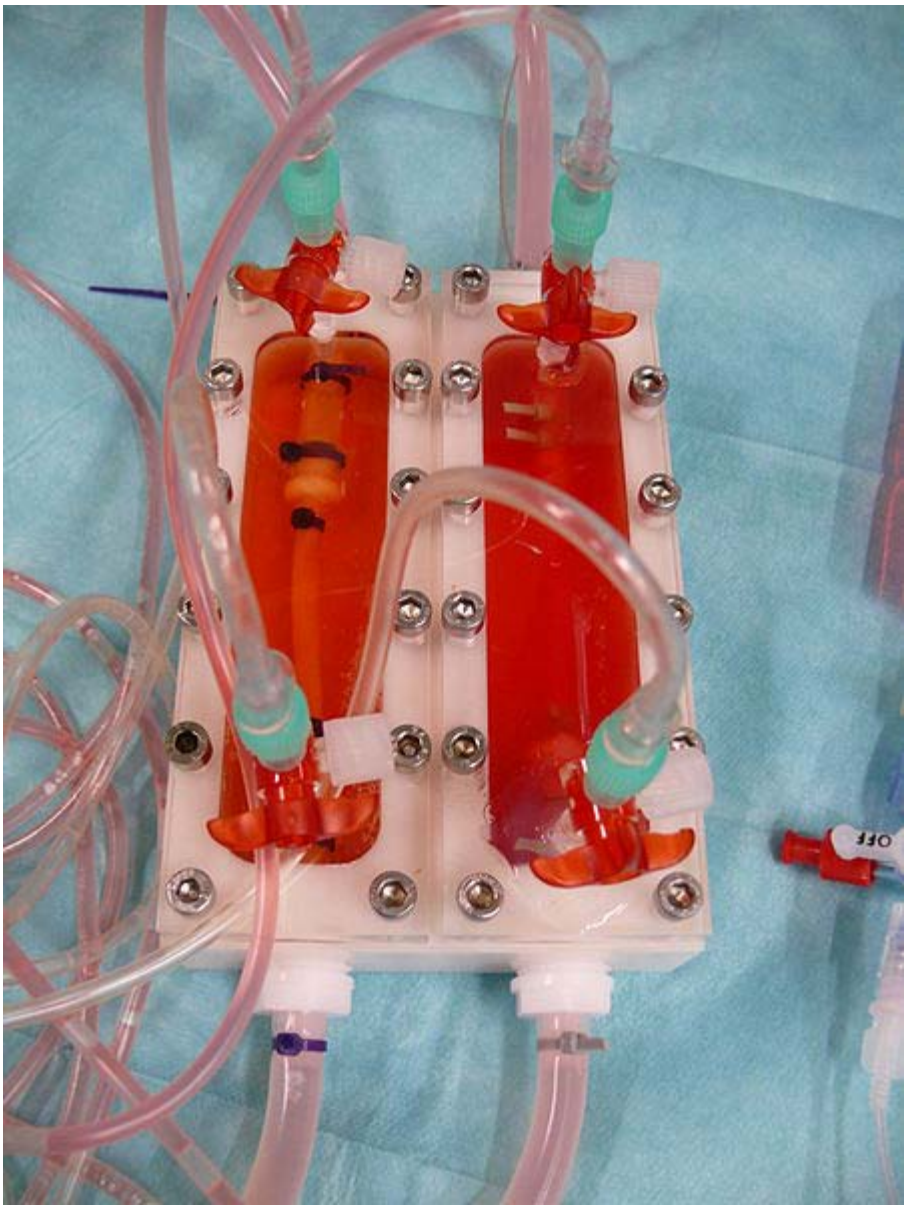
It might be possible to print organs, or possibly entire organisms. A recent innovative method of construction uses an ink-jet mechanism to print precise layers of cells in a matrix of thermoreversable gel. Endothelial cells, the cells that line blood vessels, have been printed in a set of stacked rings. When incubated, these fused into a tube.

Tissue culture

In many cases, creation of functional tissues and biological structures *in vitro* requires extensive culturing to promote survival, growth and inducement of functionality. In

general, the basic requirements of cells must be maintained in culture, which include oxygen, pH, humidity, temperature, nutrients and osmotic pressure maintenance.

Tissue engineered cultures also present additional problems in maintaining culture conditions. In standard cell culture, diffusion is often the sole means of nutrient and metabolite transport. However, as a culture becomes larger and more complex, such as the case with engineered organs and whole tissues, other mechanisms must be employed to maintain the culture, such as the creation of capillary networks within the tissue.



Bioreactor for cultivation of vascular grafts

Another issue with tissue culture is introducing the proper factors or stimuli required to induce functionality. In many cases, simple maintenance culture is not sufficient. Growth factors, hormones, specific metabolites or nutrients, chemical and physical stimuli are

sometimes required. For example, certain cells respond to changes in oxygen tension as part of their normal development, such as chondrocytes, which must adapt to low oxygen conditions or hypoxia during skeletal development. Others, such as endothelial cells, respond to shear stress from fluid flow, which is encountered in blood vessels. Mechanical stimuli, such as pressure pulses seem to be beneficial to all kind of cardiovascular tissue such as heart valves, blood vessels or pericardium.

Bioreactors

A bioreactor in tissue engineering, as opposed to industrial bioreactors, is a device that attends to simulate a physiological environment in order to promote cell or tissue growth in vivo. A physiological environment can consist of many different parameters such as temperature and oxygen or carbon dioxide concentration, but can extend to all kinds of biological, chemical or mechanical stimuli. Therefore, there are systems that may include the application of forces or stresses to the tissue or even of electrical current in two- or three-dimensional setups.

In academic and industry research facilities, it is typical for bioreactors to be developed to replicate the specific physiological environment of the tissue being grown (e.g., flex and fluid shearing for heart valve growth). Several general-use and application-specific bioreactors are also commercially available, and may provide static chemical stimulation or combination of chemical and mechanical stimulation.

Chapter- 6

Protein Engineering

Protein engineering is the process of developing useful or valuable proteins. It is a young discipline, with much research taking place into the understanding of protein folding and recognition for protein design principles.

There are two general strategies for protein engineering, *rational design* and *directed evolution*. These techniques are not mutually exclusive; researchers will often apply both. In the future, more detailed knowledge of protein structure and function, as well as advancements in high-throughput technology, may greatly expand the capabilities of protein engineering. Eventually, even unnatural amino acids may be incorporated thanks to a new method that allows the inclusion of novel amino acids in the genetic code.

Rational design of proteins

In rational protein design, the scientist uses detailed knowledge of the structure and function of the protein to make desired changes. This generally has the advantage of being inexpensive and technically easy, since site-directed mutagenesis techniques are well-developed. However, its major drawback is that detailed structural knowledge of a protein is often unavailable, and even when it is available, it can be extremely difficult to predict the effects of various mutations.

Computational protein design algorithms seek to identify novel amino acid sequences that are low in energy when folded to the pre-specified target structure. While the sequence-conformation space that needs to be searched is large, the most challenging requirement for computational protein design is a fast, yet accurate, energy function that can distinguish optimal sequences from similar suboptimal ones.

Directed evolution

In directed evolution, random mutagenesis is applied to a protein, and a selection regime is used to pick out variants that have the desired qualities. Further rounds of mutation and selection are then applied. This method mimics natural evolution and generally produces superior results to rational design. An additional technique known as DNA shuffling mixes and matches pieces of successful variants in order to produce better results. This process mimics the recombination that occurs naturally during sexual reproduction. The great advantage of directed evolution is that it requires no prior structural knowledge of a

protein, nor is it necessary to be able to predict what effect a given mutation will have. Indeed, the results of directed evolution experiments are often surprising in that desired changes are often caused by mutations that were not expected to have that effect. The drawback is that they require high-throughput, which is not feasible for all proteins. Large amounts of recombinant DNA must be mutated and the products screened for desired qualities. The sheer number of variants often requires expensive robotic equipment to automate the process. Furthermore, not all desired activities can be easily screened for.

Examples of engineered proteins

Using computational methods, a protein with a novel fold has been designed, known as Top7, as well as sensors for unnatural molecules. The engineering of fusion proteins has yielded rilonacept, a pharmaceutical which has secured FDA approval for the treatment of cryopyrin-associated periodic syndrome.

Another computational method, IPRO, successfully engineered the switching of cofactor specificity of Candida boidinii xylose reductase. Iterative Protein Redesign and Optimization (IPRO) redesigns proteins to increase or give specificity to native or novel substrates and cofactors. This is done by repeatedly randomly perturbing the backbones of the proteins around specified design positions, identifying the lowest energy combination of rotamers, and determining if the new design has a lower binding energy than previous ones. The iterative nature of this process allows IPRO to make additive mutations to the protein sequence that collectively improve the specificity towards the desired substrates and/or cofactors. Details on how to download the software implemented in Python and experimental testing of predictions are outlined in the following paper.

Chapter- 7

Protein Design & Directed Evolution

Protein design

Protein design is the design of new protein molecules, either from scratch or by making calculated variations on a known structure. The use of rational protein design techniques is a major aspect of protein engineering.

The design of minimalist computer models of proteins (lattice proteins), and the secondary structural modification of real proteins, began in the mid-1990s. The *de novo* design of real proteins became possible shortly afterwards, and the 21st century has seen the creation of small proteins with real biological functions including chiroselective catalysis, ion detection , and antiviral behaviour. There is great hope that the design of these and larger proteins will have applications in medicine and bioengineering. Recent computational redesign was capable of experimentally switching the cofactor specificity of Candida boidinii xylose reductase from NADPH to NADH.

Overview

The number of possible amino acid sequences is enormous, but only a subset of them will fold reliably and quickly to a single native state. Protein design involves identifying novel sequences within this subset, in particular those with a physiologically active native state. Physically, the native state of a protein is the conformational free energy minimum for the chain. Therefore protein design is the search for sequences which have the chosen structure as a free energy minimum. In a sense it is the reverse of structure prediction: a tertiary structure is specified, and a sequence is identified which will fold to it. Hence it is also referred to as *inverse folding*. Prion diseases like Mad Cow Disease are helpful examples of how important it is that designer proteins possess only one possible stable conformation. In Mad Cow Disease, there exists a healthy protein with a fatal weakness: there is another conformation this protein can "comfortably" take; the abnormally folded shape has very little free energy and is therefore very stable. For reasons that are not yet fully understood, this mis-folded prion protein has the ability to catalyze other proteins of its type to also adopt the mis-folded prion shape, which results in a disease-generating cascade of previously functional proteins quickly becoming misfolded. They lose the ability to perform their intended function in the new conformation, and have a tendency

to form aggregates called plaques. The buildup of these aggregates in the brain leads to progressive neuronal death, and eventually death of the entire organism.

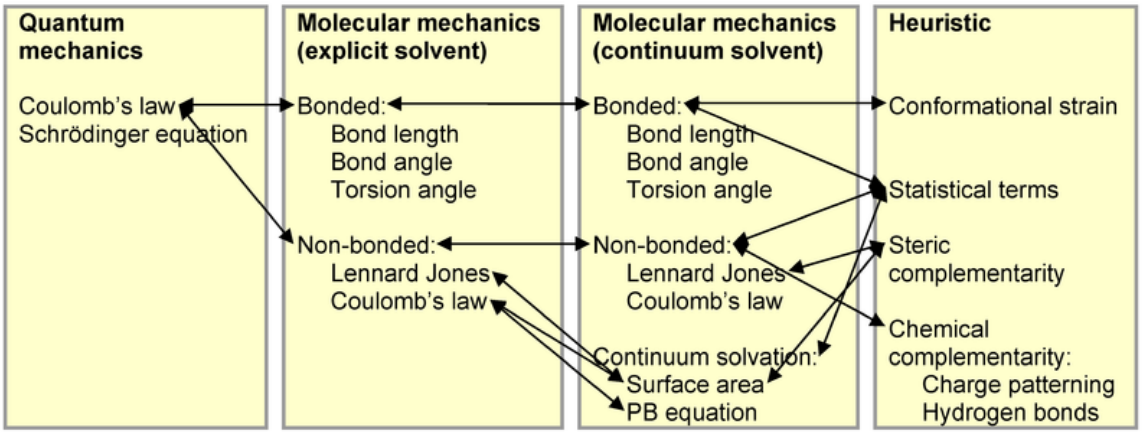
It is therefore easy to see both how important it is that a designer protein have only one possible stable tertiary structure, and that researchers exercise extreme diligence to ensure that this remains the case in all environments - especially *in vivo*.

Protein design requires an understanding of the molecular interactions that stabilize proteins in specific folded configurations; experience has shown, however, that it does not require an understanding of the dynamical process by which proteins fold.

Protein design can be accomplished using computer models, which, while simplifying the problem, are able to generate sequences that fold to the desired structure. Computational protein design algorithms search the sequence-conformation space for sequences that are low in energy when folded to the target structure. This search space is large; currently the most challenging requirement for computational protein design is a fast, yet accurate, energy function that can distinguish optimal sequences from similar suboptimal ones. Using computational methods, a protein with a novel fold has been designed, as well as sensors for unnatural molecules.

On the other hand, it is widely believed that not all possible protein structures are *designable*, which means that there are compact configurations of the chain which no sequences can fold to. In particular, conformations which are poor in secondary structures are unlikely to be designable. The designability of given structures is an issue that is still poorly understood.

Models of protein structure and function used in protein design



Comparison of various potential energy functions

Computational protein design algorithms use models of protein energetics to evaluate how mutations would affect a protein's structure and function. These energy functions typically include a combination of molecular mechanics, statistical (i.e. knowledge-based), and other empirical terms. However, the trend has been towards using more physically based potential energy functions.

Ancestral sequence reconstruction

Ancestral reconstruction techniques have been used to design proteins with putative ancient functions.

Software

Iterative Protein Redesign and Optimization. IPRO redesigns proteins to increase or give specificity to native or novel substrates and cofactors. This is done by repeatedly randomly perturbing the backbones of the proteins around specified design positions, identifying the lowest energy combination of rotamers, and determining if the new design has a lower binding energy than previous ones. The iterative nature of this process allows IPRO to make additive mutations to the protein sequence that collectively improve the specificity towards the desired substrates and/or cofactors. Experimental testing of predictions by IPRO successfully switched the cofactor preference of Candida boidinii xylose reductase from NADPH to NADH. Details on how to download the software implemented in Python and experimental testing of predictions are outlined in the following paper.

EGAD: A Genetic Algorithm for protein Design. A free, open-source software package for protein design and prediction of mutation effects on protein folding stabilities and binding affinities. EGAD can also consider multiple structures simultaneously for designing specific binding proteins or locking proteins into specific conformational states. In addition to natural protein residues, EGAD can also consider free-moving ligands with or without rotatable bonds. EGAD can be used with single or multiple processors.

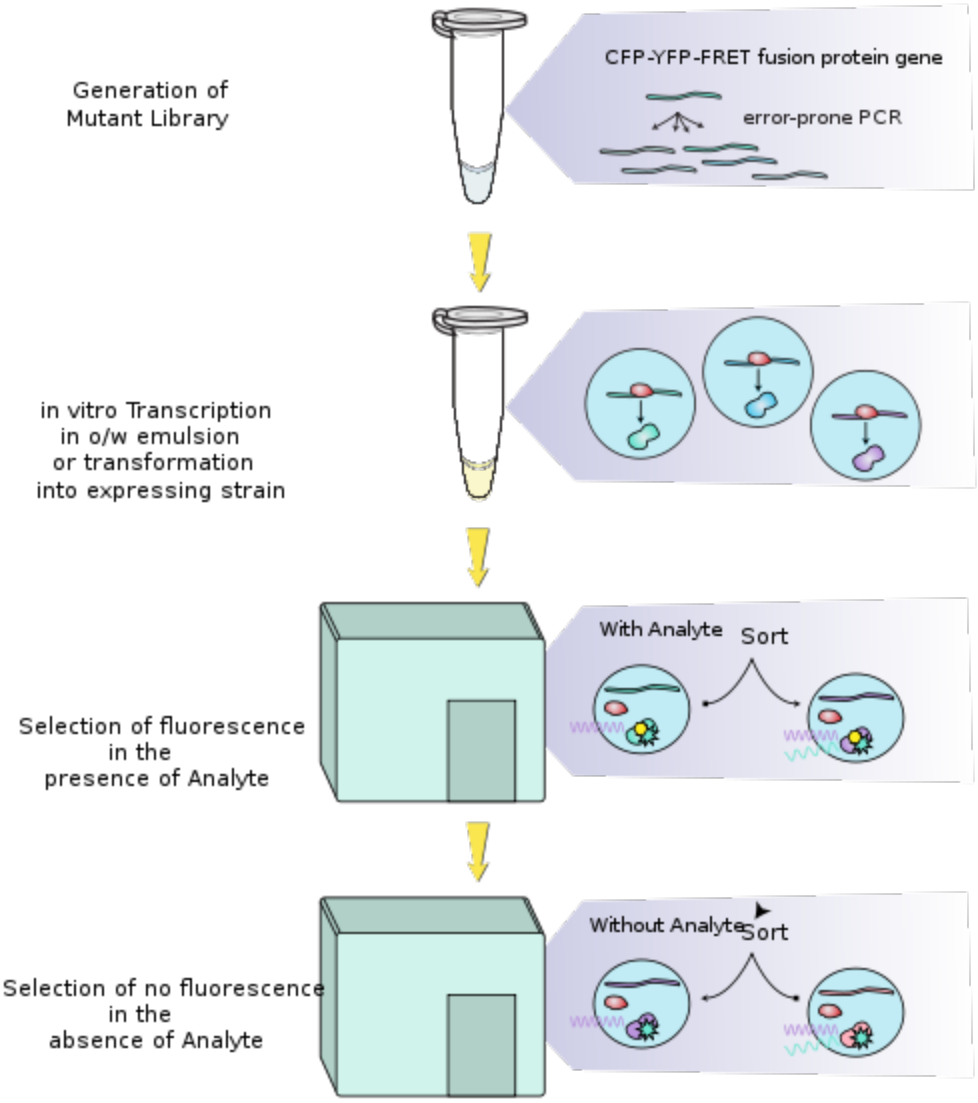
RosettaDesign. A software package, under active development and free for academic use, that has seen extensive successful use. RosettaDesign is accessible via a web server.

SHARPEN. A permissive open-source library for protein design and structure prediction. SHARPEN offers a variety of combinatorial optimization methods (e.g. Monte Carlo, Simulated Annealing, FASTER) and can score proteins using the successful Rosetta all-atom force field or molecular mechanics force fields (OPLSaa). In addition to the protein modeling library, SHARPEN includes tools for scalable distributed computing.

WHAT IF software for protein modelling, design, validation, and visualisation.

Abalone software for protein modelling and visualisation.

Directed evolution



An example of a possible round to evolve a protein based fluorescent sensor for a specific analyte using two consecutive FACS sortings

Directed evolution is a method used in protein engineering to harness the power of natural selection to evolve proteins or RNA with desirable properties not found in nature.

A typical experiment

A typical directed evolution experiment involves three steps:

1. *Diversification:* The gene encoding the protein of interest is mutated and/or recombined at random to create a large library of gene variants. Techniques commonly used in this step are error-prone PCR and DNA shuffling.

2. *Selection:* The library is tested for the presence of mutants (variants) possessing the desired property using a screen or selection. Screens enable the researcher to identify and isolate high-performing mutants by hand, while selections automatically eliminate all nonfunctional mutants.
3. *Amplification:* The variants identified in the selection or screen are replicated manyfold, enabling researchers to sequence their DNA in order to understand what mutations have occurred.

Together, these three steps are termed a "round" of directed evolution. Most experiments will perform more than one round. In these experiments, the "winners" of the previous round are diversified in the next round to create a new library. At the end of the experiment, all evolved protein or RNA mutants are characterized using biochemical methods.

Likelihood of success

The likelihood of success in a directed evolution experiment is directly related to the total library size, as evaluating more mutants increases the chances of finding one with the desired properties. Performing multiple rounds of evolution is useful not only because a new library of mutants is created in each round, but because each new library uses better mutants as templates. The experiment is analogous to climbing a hill on a landscape where elevation is a function of the desired property. The goal is to reach the summit, which represents the best mutant. Each round of selection samples mutants on all sides of the starting template and selects the mutant with the highest elevation, thereby climbing the hill. A new round samples mutants on all sides of this new template and picks the highest of these, and so on until the summit is reached.

In vivo *and* in vitro

Directed evolution can be performed in living cells (*in vivo* evolution) or may not involve cells at all (*in vitro* evolution). *In vivo* evolution has the advantage of selecting for properties in a cellular environment, which is useful when the evolved protein or RNA is to be used in living organisms, but *in vitro* evolution is often more versatile in the types of selections that can be performed. Furthermore, *in vitro* evolution experiments can generate larger libraries because the library DNA need not be inserted into cells, the currently limiting step.

Advantages

The advantage of the directed evolution approach is that the researcher need not understand the mechanism of the desired activity in order to improve it. An alternative method is rational design of site-directed mutagenesis based on X-ray crystallography data.

Uses

Most directed evolution projects seek to evolve properties that are useful to humans in an agricultural, medical or industrial context (biocatalysis). It is thus possible to use this method to optimize properties that were not selected for in the original organism. This may include catalytic activity, catalytic specificity, thermostability and many others.

Chapter- 8

Other Types of Biological Engineering

Agricultural engineering

A modern farm tractor

Agricultural engineering is the engineering discipline that applies engineering science and technology to agricultural production and processing. Agricultural engineering

combines the disciplines of animal biology, plant biology, and mechanical, civil, electrical and chemical engineering principles with a knowledge of agricultural principles. It utilizes the knowledge of engineering for making agricultural machinery.

Subfields

Wheat harvest with a combine in Denmark

Some of the specialties of agricultural engineers include:

- the design of agricultural machinery, equipment, and agricultural structures
- crop production, including seeding, tillage, irrigation and the conservation of soil and water
- animal production, including the care and processing of poultry and fish and dairy management
- the processing of food and other agricultural and biorenewable products,and food engineering.
- Bioresource engineering, which uses machines on the molecular level to help the environment.

Vodka bottling machine in Russia

History

The first curriculum in Agricultural Engineering was established at Iowa State University by J. B. Davidson in 1905. The American Society of Agricultural Engineers, now known as the American Society of Agricultural and Biological Engineers, was founded in 1907.

Pivot irrigation of cotton

Agricultural engineers

Agricultural Engineers may perform tasks as planning, supervising and managing the building of dairy effluent schemes, irrigation, drainage, flood and water control systems, perform environmental impact assessments, agricultural product processing and interpret research results and implement relevant practices. A large percentage of agricultural engineers work in academia or for government agencies such as the United States Department of Agriculture or state agricultural extension services. Some are consultants, employed by private engineering firms, while others work in industry, for manufacturers of agricultural machinery, equipment, processing technology, and structures for housing livestock and storing crops. Agricultural engineers work in production, sales, management, research and development, or applied science.

Biochemical engineering

Bioreactors for producing proteins, NRC Biotechnology Research Institute, Montréal, Canada

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 often taught as a supplementary option to chemical engineering or biological engineering due to the similarities in both the background subject curriculum and problem-solving techniques used by both professions. Its applications are used in the food, feed, pharmaceutical, biotechnology, and water treatment industries.

Biomechanical engineering

Biomechanical Engineering is a bioengineering subdiscipline which applies principles of mechanical engineering to biological systems and stems from the scientific discipline of biomechanics. Many cases are related to Biomedical engineering and Agricultural engineering.

Food engineering

Bread factory in Germany

Food engineering is a multidisciplinary field of applied physical sciences which combines science, microbiology, and engineering education for food and related industries. Food engineering includes, but is not limited to, the application of agricultural engineering and chemical engineering principles to food materials. Food engineers provide the technological knowledge transfer essential to the cost-effective production and commercialization of food products and services.

Food engineering is a very wide field of activities. Prospective major employers for food engineers include companies involved in food processing, food machinery, packaging, ingredient manufacturing, instrumentation, and control. Firms that design and build food processing plants, consulting firms, government agencies, pharmaceutical companies, and health-care firms also hire food engineers. Among its domain of knowledge and action are:

- research and development of new foods, biological and pharmaceutical products
- development and operation of manufacturing, packaging and distributing systems for drug/food products
- design and installation of food/biological/pharmaceutical production processes
- design and operation of environmentally responsible waste treatment systems
- marketing and technical support for manufacturing plants.

Topics in food engineering

In the development of food engineering, one of the many challenges is to employ modern tools and knowledge, such as computational materials science and nanotechnology, to develop new products and processes. Simultaneously, improving quality, safety, and security remain critical issues in food engineering study. New packaging materials and techniques are being developed to provide more protection to foods, and novel preservation technologies are emerging. Additionally, process control and automation regularly appear among the top priorities identified in food engineering. Advanced monitoring and control systems are developed to facilitate automation and flexible food manufacturing. Furthermore, energy saving and minimization of environmental problems continue to be important food engineering issues, and significant progress is being made in waste management, efficient utilization of energy, and reduction of effluents and emissions in food production.

Typical topics include:

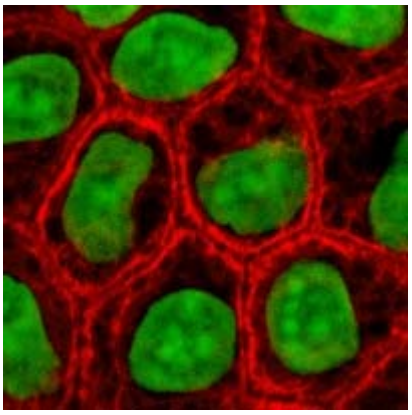
- Advances in classical unit operations in engineering applied to food manufacturing
- Progresses in the transport and storage of liquid and solid foods
- Developments in heating, chilling and freezing of foods
- Advanced mass transfer in foods
- New chemical and biochemical aspects of food engineering and the use of kinetic analysis
- New techniques in dehydration, thermal processing, non-thermal processing, extrusion, liquid food concentration, membrane processes and applications of membranes in food processing
- Shelf-life, electronic indicators in inventory management, and sustainable technologies in food processing
- Modern packaging, cleaning, and sanitation technologies

Bioprocess engineering

Bioprocess Engineering is a specialization of Biotechnology, Chemical Engineering or of Agricultural Engineering. It deals with the design and development of equipment and

processes for the manufacturing of products such as food, feed, pharmaceuticals, nutraceuticals, chemicals, and polymers and paper from biological materials.

Metabolic engineering



Cellular metabolism can be optimized for industrial use.

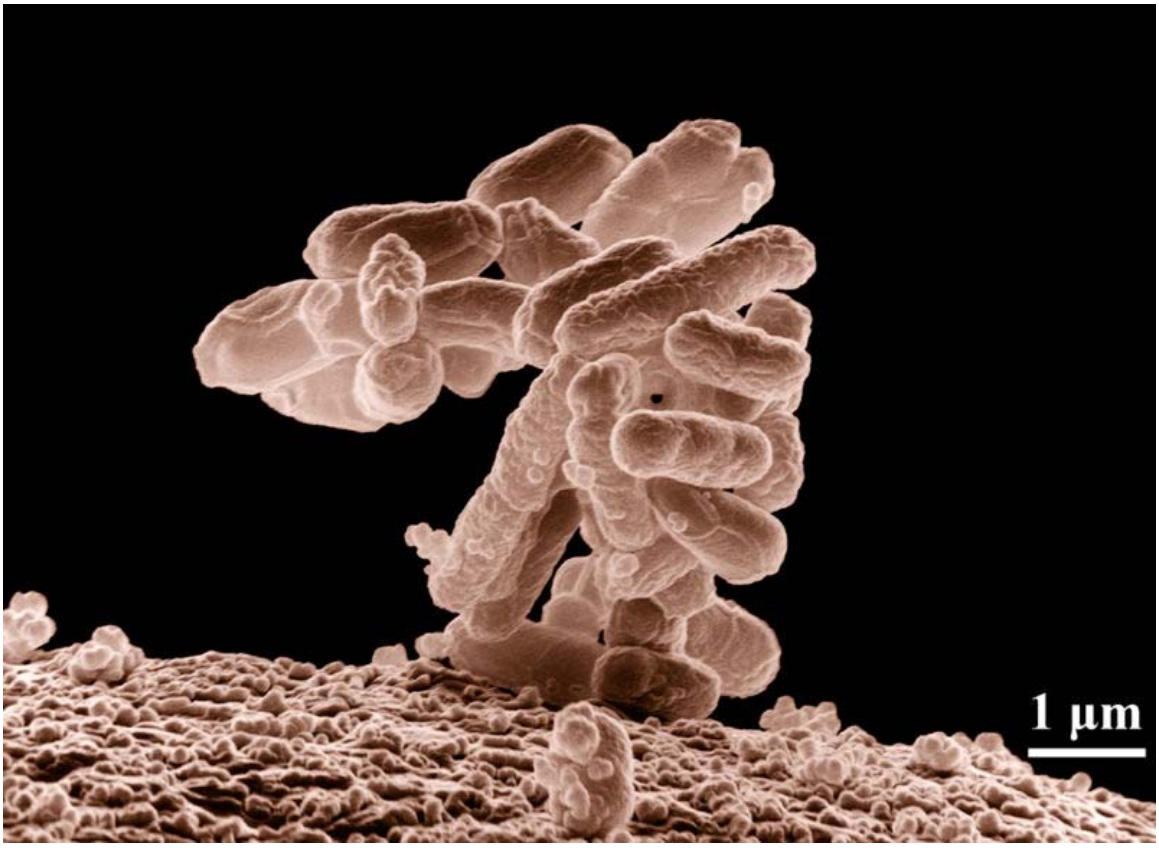
Metabolic engineering is the practice of optimizing genetic and regulatory processes within cells to increase the cells' production of a certain substance. Metabolic engineers commonly work to reduce cellular energy use (ie, the energetic cost of cell reproduction or proliferation) and to reduce waste production. Producing beer, wine, cheese, pharmaceuticals, and other biotechnology products often involves metabolic engineering.

Cells are complex systems; genetic and regulatory changes can have drastic effects on the cells' ability to survive. Therefore, trade-offs become apparent during metabolic engineering.

In addition to directly deleting and/or overexpressing the genes that encode for metabolic enzymes, the current focus is to target the regulatory networks in a cell to efficiently engineer the metabolism .

Decreasing cellular energy use

Metabolic engineering can be useful in industry. Certain industries use cells to create useful products. Producing the greatest number of those cells is a sought-after goal. The only known method of production, however, may involve oxidizing of carbon compounds. The carbon compounds may be in limited supply. Therefore, engineering cells to reproduce or proliferate more rapidly given the same amount of carbon compounds would mean greater industrial efficiency.



E. coli genes were added to the *M. methylotrophus* genome.

The role of *Methylophilus methylotrophus* in the animal feed industry is an example. *M. methylotrophus* uses methanol to produce certain proteins used in animal feed. Producing greater masses of proteins using the same mass of methanol would increase efficiency. Windass, *et al.* (1980) accomplished this by silencing genes in *M. methylotrophus* and inserting genes from *E. coli*. This example of metabolic engineering resulted in an organism capable of using a lesser mass of adenosine triphosphate to produce the same mass of glutamate.

Molecular engineering

Molecular engineering is any means of manufacturing molecules. It may be used to create, on an extremely small scale, most typically one at a time, new molecules which may not exist in nature, or be stable beyond a very narrow range of conditions.

Today this is an extremely difficult process, requiring manual manipulation of molecules using such devices as a scanning tunneling microscope. Eventually it is expected to exploit life-like self-replicating 'helper molecules' that are themselves engineered. Thus the field can be seen as a precision form of chemical engineering that includes protein engineering, the creation of protein molecules, a process that occurs naturally in biochemistry, e.g., prion reproduction. However, it provides far more control than genetic

modification of an existing genome, which must rely strictly on existing biochemistry to express genes as proteins, and has little power to produce any non-proteins.

Molecular engineering is an important part of pharmaceutical research and materials science.

Emergence of scanning tunneling microscopes and picosecond-burst lasers in the 1990s, plus discovery of new carbon nanotube applications to motivate mass production of these custom molecules, drove the field forward to commercial reality in the 2000s.

As it matures, it is seeming to converge with mechanical engineering, since the molecules being designed often resemble small machines. A general theory of molecular mechanosynthesis to parallel that of photosynthesis and chemosynthesis (both used by living things) is the ultimate goal of the field. This may lead to a molecular assembler, according to some, such as K. Eric Drexler, Ralph Merkle, and Robert Freitas, and of the potential for integrating vast numbers of assemblers into a kg-scale nanofactory.

Molecular engineering is sometimes called generically "nanotechnology", in reference to the nanometre scale at which its basic processes must operate. That term is considered to be vague, however, due to misappropriation of the word in association with other techniques, such as X-ray lithography, that are not used to create new free-floating ions or molecules.

Future developments in molecular engineering hold out the promise of great benefits, as well as great risks.

Neural engineering

Neural engineering (also known as Neuroengineering) is a discipline within biomedical engineering that uses engineering techniques to understand, repair, replace, enhance, or otherwise exploit the properties of neural systems. Neural engineers are uniquely qualified to solve design problems at the interface of living neural tissue and non-living constructs.

Overview

This field of engineering draws on the fields of computational neuroscience, experimental neuroscience, clinical neurology, electrical engineering and signal processing of living neural tissue, and encompasses elements from robotics, cybernetics, computer engineering, neural tissue engineering, materials science, and nanotechnology.

Prominent goals in the field include restoration and augmentation of human function via direct interactions between the nervous system and artificial devices.

Much current research is focused on understanding the coding and processing of information in the sensory and motor systems, quantifying how this processing is altered in the pathological state, and how it can be manipulated through interactions with artificial devices including brain-computer interfaces and neuroprosthetics.

Other research concentrates more on investigation by experimentation, including the use of neural implants connected with external technology.

History

As neural engineering is a relatively new field, information and research relating to it is comparatively limited, although this is changing rapidly. The first journals specifically devoted to neural engineering, The Journal of Neural Engineering and The Journal of NeuroEngineering and Rehabilitation both emerged in 2004. International conferences on neural engineering have been held by the IEEE since 2003, most recently from 29 April until 2 May 2009 in Antalya, Turkey 4th Conference on Neural Engineering.

Rehabilitation engineering

Rehabilitation engineering is the systematic application of engineering sciences to design, develop, adapt, test, evaluate, apply, and distribute technological solutions to problems confronted by individuals with disabilities. Functional areas addressed through rehabilitation engineering may include mobility, communications, hearing, vision, and cognition, and activities associated with employment, independent living, education, and integration into the community

While some rehabilitation engineers have master's degrees in rehabilitation engineering, usually a subspecialty of Biomedical engineering, most rehabilitation engineers have undergraduate or graduate degrees in biomedical engineering, mechanical engineering, or electrical engineering. A Portuguese university provides an undergraduate degree in Accessibility and Rehabilitation Engineering . Qualification to become a Rehab' Engineer in the UK is possible via a University BSc Honours Degree course (such as the one at Coventry University .)

The rehabilitation process for people with disabilities often entails the design of assistive devices such as Walking aids intended to promote inclusion of their users into the mainstream of society, commerce, and recreation.

The Rehabilitation Engineering and Assistive Technology Society of North America, whose mission is to "improve the potential of people with disabilities to achieve their goals through the use of technology", is one of the main professional society for rehabilitation engineers .

Rehabilitation Engineering Research Centers conduct research in the rehabilitation engineering, each focusing on one general area or aspect of disability . For example, the

Smith-Kettlewell Eye Research Institute conducts research for the blind and visually impaired . Many of the Veterans Administration Rehabilitation Research & Development Centers conduct rehabilitation engineering research

Within the National Health Service Rehabilitation Engineers (REs) are commonly involved with assessment and provision of wheelchairs and seating to promote good posture and independent mobility. This includes electrically powered wheelchairs, active user (lightweight) manual wheelchairs, and in more advanced clinics this may include assessments for specialist wheelchair control systems and/or bespoke seating solutions. Professional registration of NHS Rehab' Engineers is with the Institute of Physics and Engineering in Medicine (IPEM).