



Biotechnology & Medicine

POSTGRADUATE COURSE

“Green Technology Foresight about challenges from biotechnology and ICT”

Digit-BioTech

2019-1-BG01-KA203-062371

DIGIT~Bio~TECH



L01 SYSTEMS BIOLOGY AND OMICS TECHNOLOGY

Advanced level

AUTHOR:

VENTSISLAVA PETROVA



Contents

Contents	2
OMICS technologies towards improving the quality of life	3
OMICS Technologies and Molecular Medicine	3
OMICs technologies in diagnostics.....	4
OMICs analysis for revealing genetic architecture of common disease	9
Application of OMICs approach in disease treatment	10
OMICs technologies for disease prevention - future prospects.....	11
Application of OMICS Technologies in Toxicology	11
Toxicological studies via OMICs analysis.....	12
OMICs applications for toxicology risk assessment and regulatory submissions	16
Environmental OMICS and Biodiversity	17
Single-cell genomics	18
Omics Approaches in Industrial Biotechnology and Bioprocess Engineering	23
Omics-Guided Biotechnology.....	23
Nutri-omics Research.....	28
Genomics and nutrition	28
Transcriptomics and nutrition	30
Proteomics and nutrition	30
Metabolomics and nutrition	31
Nutrition and other OMICs	31
References	32



OMICS technologies towards improving the quality of life

OMICS TECHNOLOGIES AND MOLECULAR MEDICINE

Advances in omics technologies - such as genomics, transcriptomics, proteomics and metabolomics - have begun to enable medicine at an extraordinarily detailed molecular level. The rapidly decreasing costs of high-throughput sequencing and other massively parallel technologies, such as mass spectrometry, are enabling their use in clinical research and clinical practice. Exome and genome sequencing are already being used to aid diagnoses, particularly of rare diseases, to inform cancer treatment and progression and, in early efforts, to create predictive models of disease in healthy individuals. Numerous research efforts and companies are focusing on genome-wide profiles of genetic, gene expression and other omics data, such as the microbiome, as biomarkers for disease. These techniques have also been applied in identifying risk loci for disease, defining precise pathophysiology of the disease, as well as performing research in Occupational Environmental Health (OEH).

Ideally, different technologies would be combined both to help diagnose disease and to create a holistic picture of human phenotypes and disease. However, implementation of multi-omics data introduces new informatics and interpretation challenges. What are the ways in which integrative omics can impact medicine by helping to manage health, as well as diagnose and treat disease? In fact, to improve the treatment outcomes and reduce adverse events that matter to both the clinician and patient one of the perspective solutions is to develop the personalized medicine - customized medical treatment created to fit individuals' characteristics, needs and unique molecular and genetic profile (Fig. 1).

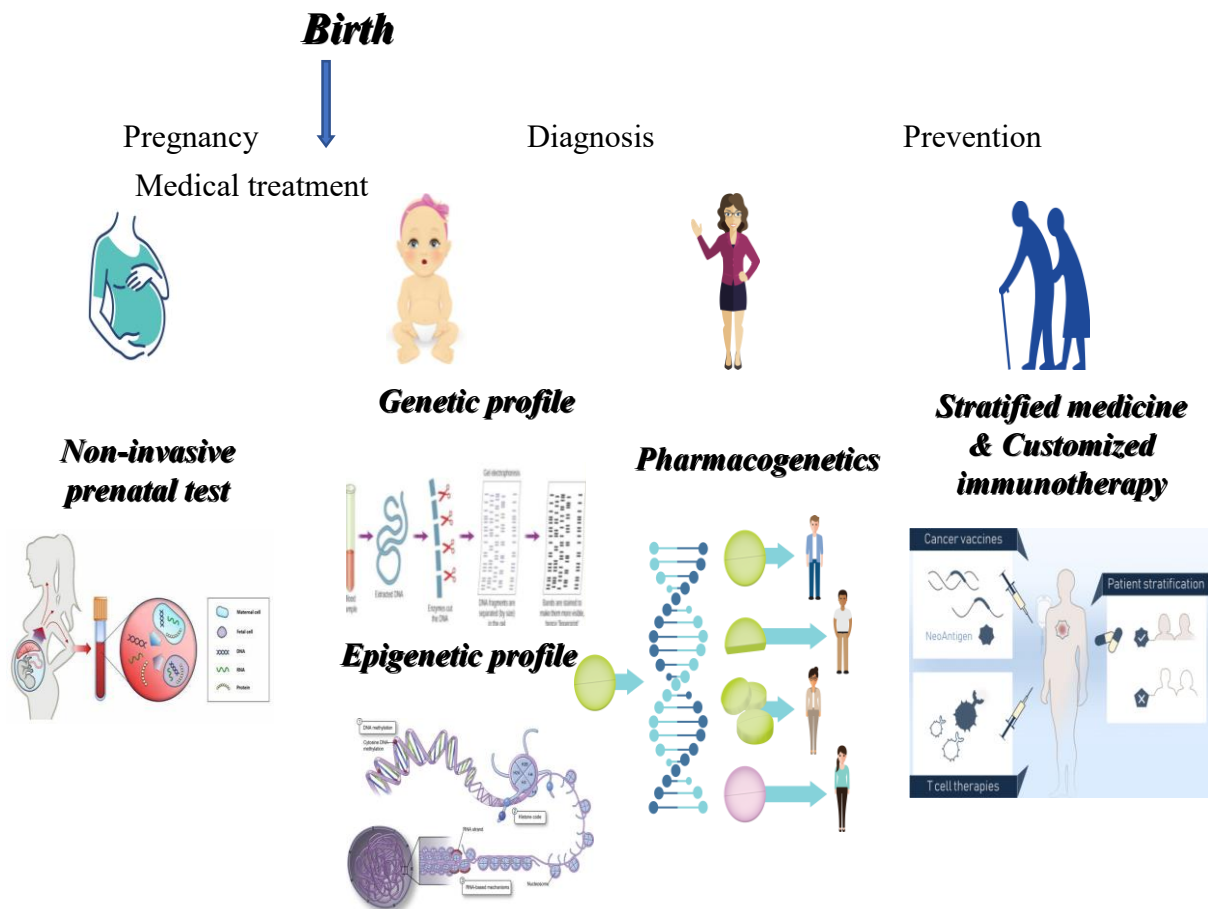


Fig. 1. Personalised medicine throughout the lifespan

1. OMICs technologies in diagnostics

PRENATAL DIAGNOSTICS

Nearly 10% of pediatric diseases and 20% of infant deaths are due to Mendelian diseases. Genetic defects can be a major threat to the baby's health. To overcome this issue, last developments in the omics technologies are employed in a person's life even before birth. Prenatal screening has become widespread



2019-1-BG01-KA203-062371

in many countries for examining not only abnormalities of a fetus but also the possible risks of diseases or abnormal conditions after birth. When a genetic predisposition exists, such as cystic fibrosis or muscular dystrophy, consulting a medical specialist or genetic diagnosis company become of great importance. The latest innovations in molecular and cellular biology allowed various genetic diagnoses for fetuses. Till recently, different invasive technologies have been applied such as amniocentesis and chorionic villus sampling. Although the procedures have been improved, there is still risk of miscarriage and serious side effects. One of the innovative attempts to avoid the side effects of conventional invasive technologies is to isolate and analyze fetal nucleated cells from maternal blood. Unfortunately, the number of fetal cells that can be isolated is about 3-5 cells per 30 ml of maternal blood. The huge jump in the field of non-invasive prenatal testing (NIPT) has been made after the advancement of omics technologies and their combination with various experimental approaches. The use of DNA amplification technology combined with NGS allowed the direct detection of the fetal DNA in maternal blood. Currently, such approach is used to determine the sex and Rh blood type, and to analyze chromosomal aneuploidies. The cell-free fetal DNA is typically released in maternal blood during the process of apoptosis of placental cells. The fragmented cell-free fetal DNA is ~200 bp long and has a half-life of ~16 min and can be detected in maternal plasma from early pregnancy (5-7 weeks). In addition to fetal DNA, fetal RNAs can also be used for NIPT. For example, fetal trisomy 21 can be diagnosed by checking the allelic ratio of *PLAC4* mRNA, produced from chromosome 21 in the placenta. If a baby receives heterogeneous alleles from the parents, an unequal allelic ratio (2 : 1) suggests trisomy.

Integrating fetal genomic, transcriptomic, proteomic, metabolomic, and epigenetic data will help pushing forward the prenatal genetic diagnostics. In this way, any genetic disorder could be identified early in the pregnancy by applying non-invasive approaches. However, some concerns occur regarding raise of serious ethical issues possibilities when advanced genomic technology are used. Therefore, further studies on the impact of new technologies on society, such as possible discrimination based on genetic information, undetermined harmful effects of nanotechnologies on humans and nature, and copyright infringements due to the development of information technology should be considered (see LO8).

LAB-ON-A-CHIP TECHNOLOGY IN DIAGNOSTICS

Rapidly after the development of micro-electro-mechanical systems (MEMS), the potential use of these miniaturized platforms for various applications has been revealed. During the past few decades, interest in biological or biomedical MEMS (BioMEMS) has been significantly increased and it has found widespread applications in a various area life science including diagnostics, therapeutics, drug delivery, biosensors and tissue engineering. These integrated systems are also known as “lab-on-a-chip” (LOC) or “micro-total analysis systems” (μ TAS) and provide a solid way for miniaturization, integration, automation, and parallelization of chemical processes.



Lab-on-a-chip devices integrate multiple laboratory functions on a single chip (from few square millimeters to a few square centimeters in size). These platforms provide complex chemical and/or biological analyses which can offer cheaper, faster, controllable and higher performance of bio-chemical assays at a very small scale when compared with conventional laboratory tests. They are capable of handling extremely small fluid volumes down to less than a few picoliters (few micro-droplets of whole blood, plasma, saliva, tear, urine or sweat). This fact is very important in many clinical trials where usually very small volumes of patient samples are available. Moreover, the automation with eliminating the human interfering parameters can increase the reliability in the analysis.

LOC has been used in different applications, such as: DNA extraction and purification, PCR, qPCR, molecular detection, electrophoresis, etc.

LOC for DNA Extraction and Purification: Often for diagnostic assays nucleic acid have to be purified from cells. The process of DNA extraction generally includes two steps: cell lysis by disrupting cellular and nucleus membrane, and DNA purification from membrane lipids, proteins, and RNA. Cell lysis on LOC devices can be achieved through several types of lysis methods: chemical, thermal, ultrasonic, electrical, mechanical or some other type of lysis. The well known DNA purification methods through extraction columns, using the DNA adsorption on silica beads under certain buffer conditions, are also adaptable to microfluidic techniques.

LOC Devices for PCR, qPCR, and Molecular Detection: After DNA extraction, the most frequent analysis is the PCR. The importance of PCR in genomic analysis led to the development of numerous LOC devices. Miniaturization of volume and the high surface to volume ratio defines rapid thermal transfer and more accurate PCR. This analysis requires a post-analysis and amplicons' size detection, which generally is carried out by electrophoresis. With the introduction of LOCs, DNA electrophoresis was one of the first molecular processes that was integrated on a chip. This miniaturization diminishes even more the overall process time and reduces the reagent consumption.

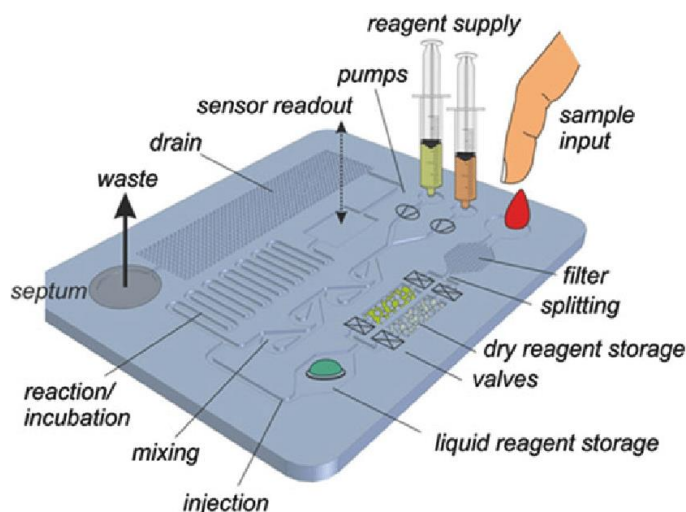


Fig. 2. Lab-on-a-Chip device

qPCR is another technique that was modified to LOC devices. Using ultra-fast pressure controller and fluorescence reader, ultra-fast qPCR microfluidic system had been developed for the molecular detection of diseases like Anthrax and Ebola. The detection efficiency is identical to commercial systems and results are achievable in less than 8 minutes being 7-15 times faster than traditionally used ones. Other advanced field is digital microfluidics that deals with emulsion and droplets within LOC devices. Ultra-low quantity of DNA can be captured within droplets, and detection limits can be enhanced with one copy number detection within LOC droplet qPCR.

Genomic Application: Due to the numerous sequencing projects, such as the Human Genome Project, the field of genomics gained a tremendous attention in recent years. Next-generation sequencing techniques of DNA which are currently under development include sequencing-by-hybridization (SBH), nanopore sequencing, and sequencing-by synthesis (SBS), the latter of which includes many different DNA polymerase dependent strategies. Using LOC approach allows the drastic reduction in cost and duration of high-throughput sequencing.

When studying the complex DNA samples expression an integration of multiple biosensors in connection with DNA microarrays is required. The most essential characteristics of these LOCs are miniaturization, speed, and precision. This technology offers vast possibilities for rapid multiplex analysis of nucleic acid samples, including the diagnosis of genetic diseases, detection of infectious agents, measurements of differential gene expression, drug screening, or forensic analysis.

Biochemical Applications: LOC strategy is also applicable in coupling enzymatic and immunological assays on a single-channel microfluidic device. Typical example is the simultaneous measurement of glucose and insulin. Key for the effective realization of such glucose/insulin monitoring is the integration of relevant sample pretreatment/cleanup procedures essential for whole-blood analysis.



This innovative LOC approach can be applicable to the incorporation of other analytes and additional sample handling steps, as is desired for creating miniaturized clinical instruments.

Proteomics: Proteomics is one of the great scientific challenges in the post-genome era. LOC devices are useful for creating advanced techniques to answer complex analytical problems, such as proteome profiling. LOC devices could be applied in four key areas of proteomics: chemical processing, sample preconcentration and cleanup, chemical separations, and interfaces with mass MS. LOC being a miniaturized device could be used for separation and detection of proteins. This process is characterized with less reagent consumption, easy operation, and very fast analysis. Another benefit is that the data from the LOC devices can easily be compared to those obtained using 2D-PAGE. In order to quantitate proteins a LOC MS protein profiling device is developed. Some authors describe LOC devices that are used for direct infusion into the mass spectrometer, including Capillary Electrophoresis (CE) separation, on-chip sample digestion, and infusion or CE before MS analysis. Researchers use electrospray ionization MS and focus on the interface design between LOC and the mass spectrometer.

Biosensors: For detecting target analytes LOC biosensors are also developed. Such devices intricately link a biological recognition element with a physical transducer converting the biorecognition event into an electrical signal. There are two types of LOC biosensors: bioaffinity and biocatalytic devices. Bioaffinity devices are based on selective binding of the target analyte to a surface-enclosed ligand partner (e.g., antibody, oligonucleotide). For example, in hybridization biosensors, there are immobilized single-stranded (ss) DNA probes onto the transducer surface. When a duplex is formed it can be detected following the association of an appropriate hybridization indicator. On the other hand, in biocatalytic devices, an immobilized enzyme is used for recognizing the target substrate. For example, sensor strips with immobilized glucose oxidase for monitoring of diabetes (for more information see LO3).

Cell Research: Using LOC devices a small-scale experiment with cells could also be performed. The single cell is positioned within the microchannel in a predetermined location, making its handling and manipulation easy. The microsystem allows handling small volumes of fluid containing small quantities of analyte. LOC systems also provide precise control of the local environment around the cell, monitoring the physical or chemical nature of the surface to which the cell adheres, the local pH, and temperature. When the cell is to be exposed to a different type of stimuli, numerous and often complex fluid-handling components can be used. If the cell is a subject of further analysis, lysis and necessary analytical methods could also be introduced onto the same platform. Thus, sample loss or dilution is avoided, which would inevitably occur if multiple devices were used.

Drug Development: The industry needs for development of new drugs and to predict their potential behavior in animals and cells triggers other analytical applications of LOC systems: e.g., analytical systems to monitor and optimize the production of protein drugs such as therapeutic antibodies; assays based on primary human cells that could predict performance in human clinical trials and others. These LOC systems are proven to be highly reproducible, easily manipulated, and could be used routinely.



2. OMICs analysis for revealing genetic architecture of common disease

Identifying genetic markers that are associated with a specific disease feature is key issue when estimating the disease risk. As the genetic information of an individual remains mostly unchanged, the mutated sequences can be reliable markers for certain diseases. In this regard one of the important applications of omics technology is the genome-wide association studies (GWASs). They can identify common genetic variants, generally single nucleotide polymorphisms (SNPs), associated with certain disease features. In general, SNP information is collected from two groups, a patient group and a healthy control group. Then it is statistically analyzed, and the SNPs associated with the specific disease characteristics of the corresponding patient group re identified.

For example, variations in the apolipoprotein E (APOE) gene are a well-known genetic factor that is correlated with the late-onset Alzheimer's disease (LOAD). After the assessment of 502,627 SNPs in 1086 AD patients, it was demonstrated that rs4420638 on chromosome 19 is the strongest marker differentiating AD patients from the control group. Other important success is achieved during investigation of hereditary cancers. BRCA1 and BRCA2 genes are one of the most important genetic markers for hereditary breast and ovarian cancers. These genes encode tumor suppressor proteins, which help repairing damaged DNA through homology directed repair. Some mutations in BRCA1 and/or BRCA2 significantly raise the risk of developing breast and/or ovarian cancers. For BRCA1, two mutations were detected: 185delAG and 5382insC. Approximately 55-65% of women who inherit one of the harmful BRCA1 mutations and around 45% of women who carry the BRCA2 mutation have developed breast cancer by the age of 70. Numerous other disease markers such as autosomal-dominant colorectal cancer, Li-Fraumeni multi-cancer syndrome, mucopolysaccharidosis IV, and hypertrophic cardiomyopathy, have also been successfully identified using NGS.

However, for the most common diseases such as diabetes, obesity, schizophrenia and autism, a combination of multiple genetic and environmental factors is responsible. A growing number of evidence indicates that genetic markers commonly explain only a part of the total trait variation. One of the missing crucial factors are the epigenetic variations. Various epigenetic modifications have been reported to be associated with the early stage of different cancers (abnormal growth of a tumor). Moreover, it is well known that colorectal cancer is more frequently identified in men than in women. That is why it has been suggested that estrogen may help suppress colorectal cancer. In addition, the epigenetic profiles of adjacent mucosa, and non-adjacent mucosa samples of 95 colorectal cancer patients have revealed that the promoter of the MGMT, a DNA repair gene, was frequently methylated. Up to now, thousands of genomic loci have been identified and associated with various human diseases. However, the difficulties arise when these genes have to be characterized in the context of the molecular pathophysiology and the corresponding interacting genes and pathways.



3. Application of OMICs approach in disease treatment

The main aim of personalized medicine is to decide on the most effective treatment option for an individual. In this regard, the simultaneous quantification of multiple protein markers can play an important role in subtyping tumors and selecting optimized therapies for each subtype. High-throughput, single cell-based protein quantification techniques, such as CyTOF, can be excellent alternatives for immunohistochemistry-based methods.

For example, in the last decade a new approach for treating cancers has emerged - allowing the patient's immune system combat his or her cancer, called adoptive cell transfer (ACT). Glioblastoma multiforme (GBM) is the most frequent and aggressive type of malignant brain tumor. Although GBM has been treated with surgical removal, radiation treatment, and chemotherapy methods, there have been no noticeable effects. Over the past 20 years, numerous clinical trials have been performed to use the immune system to cure cancers such as GBM. Among numerous ACT approaches, particular attention has been paid on the dendritic cell (DC)- and T cell-based vaccines. They can penetrate and induce anti-tumoral response in the brain and show great potential for effectively killing various cancers including the malignant and advanced types. Autologous DCs, obtained from tumor tissue or in the form of a tumor lysate of a patient, can be grown *in vitro* in the presence of tumor-associated antigens (TAAs), a method called 'pulsing'. The pulsed, autologous DCs are reintroduced into the patient to stimulate specific, cell-mediated, anti-tumoral cytotoxicity against GBM and other malignant cancers. More revolutionary approaches, e.g. engineering specific immune cells using zinc-finger nucleases (ZFNs) and CRISPR technology, have also been attempted.

Every year, thousands of patients undergo a transplantation of organ or hematopoietic stem cells. However, mortality among such patients remains very high. A standard procedure for matching donors with recipients involves human leukocyte antigen (HLA) typing. However, it becomes clear that non-HLA factors can also considerably affect prognosis and development of graft-versus-host disease (GVHD). In order to overcome this, many omics applications can be used to determine optimal donor–recipient matches, as well as to examine different markers of rejection. For example, free DNA sequencing can be used to assess the severity of organ rejection as well as to simultaneously detect the eventual presence of viral DNA as a marker of infection. Additional omics data, such as RNA or protein expression, may also be utilized to evaluate the donor–recipient compatibility. Thus, integration between several omics' technologies may be used as a useful tool for transplant biology.

Furthermore, the microbiome has been associated with many common human diseases. Whereas causality is simple in genomic data, where DNA influences phenotypes, it is more difficult to unravel whether microbiome composition is a cause or effect of disease. Nevertheless, it is evident that patients with inflammatory bowel disease, type 2 diabetes and obesity, have different microbiome profiles from those of healthy controls. In addition, the microbiome has a great influence on immune function, which in some cases has been putatively causally related to disease in animal models. As our understanding of the microbiome advances, integrative assessment of this and other omics technologies data will help for our better understanding of human disease. Recently, it has been shown that human genetic profiles



2019-1-BG01-KA203-062371

influence overall gut microbiota composition. Additionally, interactions between human genetics and microbiomes have been shown to influence disease manifestation. Similarly, metabolic signaling between hosts and their microbiomes has become an area of active research, and there is increasing evidence that a number of metabolites secreted from gut bacteria may play a role in human disease. Therefore, it is obvious that integrated analysis across genome, metabolome, microbiome and other omics profiles will provide means for managing health and combating disease.

4. OMICs technologies for disease prevention - future prospects

Other important goal of personalized medicine is to prevent the development of various diseases before their onset. Current preventive treatments are limited to surgery - removing a tissue or an organ if a person possesses relevant cancer markers. However, this approach is not applicable to diseases like Alzheimer, Huntington's disease, and congenital muscular dystrophy. One of the recent approaches to address such issues is the nutrigenomics-based diet treatment. Omics profiling can be an effective approach in detecting large-scale or pathway-level changes, can show patient-specific trends and add statistical support through repeated measurements.

APPLICATION OF OMICS TECHNOLOGIES IN TOXICOLOGY

The use of omics tools to evaluate environmental health status is becoming more and more important in environmental management and risk assessment. Omics permit connecting molecular events and adverse effects with biological levels of organization relevant for risk assessment schemes. The analysis of the RNA transcripts level or cellular proteomics have already gained particular interest. These studies helped in better understanding the interactions between the stress factors and the organism, but also to unravel the modes of action (MoA) of various toxic substances. Therefore, from a toxicological point of view, omics techniques allow to effectively and accurately generate important information on substance-induced molecular perturbations in cells and tissues that are associated with adverse outcomes.

Cellular regulatory pathways involve a range of different (bio)molecules, which are characterized with different physicochemical properties and display complex non-linear interactions. A single-omics technique can measure biomolecules of a specific type, RNAs or proteins, and frequently these are not even the entirety of biomolecules of one type but a smaller subset of them. For example, the detection of short and long RNAs, or short peptides and proteins requires different transcriptomic or proteomic approaches. Only the use of multi-omics, also known as cross-omics approaches, permits to directly identify a significant fraction of a pathways' response to chemical exposure. Thereof, utilizing multi-omics strategies for toxicological research questions has greater importance.



2019-1-BG01-KA203-062371

Such system toxicology approach, integrating classical toxicology with quantitative analysis of large molecular networks and functional changes occurring across multiple levels of biological organization, is promising in analyzing the full spectrum of xenobiotics toxicity. A systems toxicology approach includes the following elements:

- Absorption and distribution of the xenobiotic within the biological system.
- Transformation of the xenobiotic and generation of toxic and/or non-toxic intermediates.
- Interaction of the xenobiotic and/or its products with the cellular targets.
- Modifications in the cellular molecules including genes, proteins, and lipids.
- Structural manifestations of target organ toxicity.
- Functional manifestations of toxicity, and
- Elimination of the xenobiotic and/or its intermediates from the biological system.

1. Toxicological studies via OMICs analysis

For better understanding the cellular response to chemical exposure, studies should be focused on different levels: transcriptomics, proteomics, phosphoproteomics, and metabolomics. Epigenomics should be considered as well for specific cases.

Transcriptomics. Transcriptomics main aim is the comprehensive detection of RNAa in the cell. A pathway response in transcriptomics is primarily detected via a known set of target genes that are found differentially expressed. The techniques applied to determine changes in gene expression in response to exposure to a xenobiotic include: northern hybridization, quantitative real time PCR (QRT-PCR), subtractive hybridization, serial analysis of gene expression, microarray analysis, and next generation sequencing. Depending on the purpose of the study, one or more of these techniques may be applied to define either partial or global expression profiles in the tested biological sample. However, the most commonly used gene expression profiling techniques are QRT-PCR, microarray analysis, and NGS. If the aim is to investigate the expression profile of a single or a limited number of transcripts, QRT-PCR analysis is the method of choice. QRT-PCR analysis is a relatively simple technique that includes the reverse transcription of mRNAs followed by PCR amplification of the resulting cDNAs using primers specific for the targeted gene as well as for one or more house-keeping genes. The amount of the PCR amplified gene products are then quantified. As QRT-PCR can accurately determine the expression of an individual or limited number of transcripts, it has some limitations from a system toxicology perspective. In order to study the systems-level toxicity of a given xenobiotic, it is necessary to employ a high throughput techniques that is capable of determining expression levels of the entire transcriptome. That is why the microarray and next generation sequencing are the most popular global transcriptome analysis techniques applied in toxicology research.

The microarray technique represents a major breakthrough analyses in the post-human genome sequencing era. Like Southern hybridization technique, the microarray approach is grounded on the



2019-1-BG01-KA203-062371

ability of DNA molecules to bind or hybridize in a nucleotide sequence-specific manner. Microarray can be considered as a high throughput northern blot hybridization in which almost all the genes that are expressed in a biological sample at a given time can be detected simultaneously. Therefore, it allows to detect small differences in the global gene expression profiles in a given biological sample in response to xenobiotic(s) exposure. The microarray technique involves the isolation of high quality RNA, reverse transcription of the mRNA to synthesize cDNA, cRNA synthesis and labeling to generate “probes”, hybridization of the “probes” with “targets” present on the microarray, detection of the signal intensity of the hybridized probe-target complex, and analysis of the resulting data to determine the levels of expression of the genes of interest. Microarray-based analysis has been used in different areas such as environmental toxicology, drug development, and occupational toxicology. The data obtained provides a snapshot of all the genes whose expression levels are affected in response to xenobiotic exposure.

Next generation sequencing (NGS) is the most recently developed global transcriptome analysis. This technique can be applied to determine a global gene expression profile in RNA obtained from different biological samples. The key steps involved in NGS are RNA isolation, removal of abundant RNA molecules such as ribosomal RNA and globin RNA, preparation of sequencing libraries, PCR amplification, and sequencing of the libraries. The resulting data is analyzed, and the quantity of the individual transcripts is determined.

Proteomics: Transcriptomics is often the first choice for systems-level investigations as numerous well-established measurement methods have been developed during the years. However, protein alterations can be considered to be closer to the relevant functional impact of a studied stimulus. Although mRNA and protein expression are tightly related through translation, their correlation is limited. The mRNA transcript levels only explain about 50% of the variation of protein levels. This is due to the additional levels of protein regulation including their rate of translation and degradation. Moreover, the regulation of protein activity does not stop at its expression level but is often further controlled through posttranslational modifications. From toxicology point of view such examples for important post-transcriptional regulation include: the tight regulation of p53 and hypoxia-inducible factor (HIF) protein-levels and their rapid post-transcriptional stabilization, e.g., upon DNA damage and hypoxic conditions; the regulation of several cellular stress responses (e.g., oxidative stress) at the level of protein translation; and the regulation of cellular stress response through protein phosphorylation cascades. The most often applied proteomics analyzes being of key importance for toxicology include: 2D polyacrylamide gel electrophoresis, gel-free liquid chromatography, mass spectrometry and analysis of the posttranslational modifications.

2D polyacrylamide gel electrophoresis (2DGE) is used to study perturbations on the proteome based on changes in protein expression. The 2DGE technique relies on the separation of proteins based on their pH (charge) as well as their size. It has the capability to separate and visualize up to 2000 proteins in one gel. The first dimension, which is known as isoelectric focusing (IEF) separates the proteins by their isoelectric point (pI). The second dimension further separates the proteins by their mass. State-of-the-art image acquisition and analysis software allow the comparison of control and treated samples helping to identify the differentially regulated proteins by their relative intensity. A variant of 2DGE is



2019-1-BG01-KA203-062371

difference gel electrophoresis (DIGE) which is based on labeling of proteins with fluorescent cyanine dyes (Cy2, Cy3 and Cy5). Although 2DGE is a powerful tool to identify many proteins, the approach has its limitations. The main constraint is that not all proteins can be separated by IEF, such as membrane, basic, small (< 10 kDa) and large (> 100 kDa) proteins. The second limitation is that less abundant proteins are often masked by the abundant proteins in the mixture.

Other frequently used techniques are the gel-free liquid chromatography mass spectrometry (LC MS/MS). The LC approach employs the differences in the physiochemical properties of proteins and peptides, i.e., size, charge, and hydrophobicity. 2D-LC can be applied to fractionate protein mixtures on two columns with different physiochemical properties and maximize the separation of proteins and peptides in complex mixtures. Mass spectrometry is widely believed to be the key technology platform for toxicoproteomics. Its main advantages include unsurpassed sensitivity, improved speed and the ability to produce high throughput datasets. Due to the high precision of MS, in tissues and biological matrices can be detected peptides in the femtomolar (10^{-15}) to attomolar (10^{-18}) range. This is extremely beneficial in comparative analysis where simultaneous analysis of control and treated samples are carried out. Thus, comprehensive understanding of how stimuli affect the proteome, and the subsequent identification of potential biomarkers can be achieved.

As system biology requires accurate quantification of a particular set of peptides/proteins across multiple samples, targeted approaches for biomarker quantification have been also developed. Such technique is the selected reaction monitoring (SRM). SRM measures peptides produced by the enzymatic digestion of the proteome in triple quadrupole MS. An SRM-based proteomic experiment starts with the selection of a list of target proteins, derived from preceding experiments or data search such as a pathway map or literature. This step is followed by 1) selection of the proteotypic target peptides that optimally and uniquely represent the protein target, 2) selection of a set of suitable SRM transitions for each target peptide, 3) detection of the selected peptide transitions in a sample, 4) optimization of SRM assay parameters, and 5) application of the assays to the detection and quantification of the proteins/peptides. The major advantages of the SRM technique are: 1) possibilities to monitor tens to hundreds of proteins during the same run, 2) possibilities for absolute and relative quantification, 3) highly data reproducibility, and 4) yielding absolute molecular specificity. However, some limitations of this technique also exist: 1) only a limited number of measurable proteins can be included in the same run, and 2) even with its high sensitivity it cannot detect all the proteins present in an organism.

Another MS-based targeted approach known as parallel reaction monitoring (PRM) has also been employed. It is centered on the use of next-generation, quadrupole-equipped high-resolution and accurate mass instruments (mainly the Orbitrap MS system). This technique is closely related to SRM but allows for the measurement of all fragmentation products of a given peptide in parallel.

Other key approach is the analysis of the posttranslational modifications (PTMs) occurred due to the toxic exposure. They represent an essential mechanism for regulating the cellular proteome. PTMs are chemical modifications that have an important role in regulating activity, localization and interactions of cellular biomolecules. The identification and characterization of proteins and their PTM sites are very



important to the biochemical understanding of the PTM pathways. This knowledge provides deeper insights into the possible regulation of the cellular physiology triggered by PTM. Some examples of PTMs include phosphorylation, glycosylation, ubiquitination, nitrosylation, methylation, acetylation, lipidation and proteolysis. During the past decade, MS-based proteomics has proven to be a powerful tool for the identification and mapping of PTMs. Moreover, the MS-based approaches took great advantage from the progress in MS instrumentation making the analysis more sensitive, accurate and with higher resolution for the detection of less abundant proteins.

Metabolomics: The metabolome is different to the other omics analysis, as it is dealing with a collection of chemically highly heterogeneous molecules. The metabolome is typically defined as the complete balance of all small molecule metabolites (< 1500Da) found in a specific cell, organ or organism. Thanks to initiatives like the Human Metabolome Project, the endogenous metabolome of human and animal model organisms has been mapped to a large extent. Metabolome measurements employ either nuclear magnetic resonance (NMR)-based detection or gas or liquid chromatography hyphenated to MS. NMR based approaches have the capacity to quantify the studied metabolites and constitute the gold standard for the structural elucidation of unknown metabolites. MS-based approaches surpass NMR in terms of sensitivity and can be run in an untargeted or targeted manner.

Metabolomics differs from proteomics and transcriptomics in several aspects:

- (i) detects the response at very different levels of a pathway, and
- (ii) simultaneously capturing molecules of a pathway that respond on extremely different time scales.

The progression of metabolomics has made it routinely and highly useful in many toxicology-related studies. It has remarkable potential in screening drug-induced cellular or organ toxicity. Rapid assessment of biological samples, together with mathematical and statistical analysis (chemometrics) of obtained data serves as a powerful tool for drug safety assessment. For example, if a drug or chemical substance can cause hepatic toxicity by inducing cellular oxidative stress, metabolomics analysis of the drug-exposed biological sample can provide direct information about metabolites that are markers of the oxidative stress. It can also identify metabolites that are considered as known biomarkers of hepatic injury, thus suggesting hepatic pathology. In addition, metabolomics analysis can also provide information about metabolites that are indirectly associated with toxicity.

Metabolomics has several advantages over conventional clinical pathology assessment. For instance, a study examining an unknown compound has shown that this compound increases serum cholesterol levels. Upon the utilization of metabolomics, it has been observed that, apart from cholesterol, the same compound also raises several phytosterols, indicating that the higher cholesterol is a result of the sterol absorption in the gut. Such findings clearly indicate that metabolomics has a much higher potential in terms of identifying exact toxicological endpoints.



2019-1-BG01-KA203-062371

Epigenomics. Main focus of epigenomics is to study the chemical modifications of the DNA and proteins organizing the three-dimensional structure of genomic DNA. Nowadays, DNA methylation is assessed by a modified genome sequencing approach (Methylome-seq). However, this approach still requires high coverage associated with still significant sequencing costs. That is why the targeted microarray-based approaches are most frequently used. DNA methylation includes two different chemical modifications with distinct functional consequences - 5-methylcytosine and 5-hydroxymethylcytosine. Histone modifications are analyzed using Chromatin-immuno-precipitation method applying antibodies specific for the targeted modification followed by sequencing (ChIP-seq). Typically, to get a comprehensive picture, several modifications need to be detected. That requires performance of several ChIP-seq analysis simultaneously. As ChIP-seq needs more sample material than the other epigenomic approaches, such a strategy may be hard to implement in toxicological studies. ATAC-seq is an alternative approach that does not investigate chemical modifications directly, but rather one of the modifications' main consequence, DNA accessibility.

However, knowledge on the involvement of regulatory pathways and specific epigenetic modifications is still scarce. Epigenomics is thus of limited value when a pathway-based data integration approach is used. However, epigenomic data have proven highly valuable for trans-generational studies or when trying to predict long-term effects of chemical exposure based on omics data of short-term exposure.

2. OMICs applications for toxicology risk assessment and regulatory submissions

In the last decades, 'omics technologies have been extensively used in research and it was demonstrated that they are capable to provide a profound insight into the biochemistry and physiology of the cell and any harmful effect of xenobiotics. This has led to an enthusiastic approval by research toxicologists. Hopes were expressed that 'omics technologies would provide the tools to identify a set of biomarkers of adverse effects and their modes-of-action. Thus, the prediction of human effects during substance hazard assessment will be improved and a contribution will be made to the development of alternative methods to animal testing. Despite this, the translation of 'omics into the regulatory domain remains at best cautious.

The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) has, therefore, organized a series of workshops to evaluate the possibilities and challenges of omics techniques in chemical risk assessment. The most recent workshop concluded that omics approaches contribute to answering relevant questions in risk assessment including:

- (i) the classification of substances and definition of similarity,
- (ii) the elucidation of the mode of action of substances, and
- (iii) the identification of species-specific effects and the demonstration of human health relevance.



2019-1-BG01-KA203-062371

However, a need was identified to increase the reproducibility of omics data acquisition and data analysis, as well as to define the best practices. Furthermore, the regulatory use of any test method is closely related to the specific legal framework that is relevant for the substance under investigation. Considering the REACH Regulation (EP and Council of the EU, 2006), “omics-based data could potentially be used for regulatory submissions”. To use omics in REACH dossiers, the REACH Regulation lists different requirements: specific standard information that cover specific physico-chemical, toxicological and ecotoxicological limits. These data are evaluated during hazard and risk assessment, and they are analyzed to determine risk management alternatives, respectively. The application and integration of omics tools may be useful in different levels of regulatory hazard identification and assessment contributing to:

1. Classification and labelling (C&L) of substances.
2. Weight-of-evidence (WoE) approaches to elucidate the MoA of the substance under investigation.
3. Substantiation of chemical similarity.
4. Determination of points-of-departure (PoDs) for hazard assessment.
5. Demonstration of species-specific effects and human health relevance.

The fast development of omics technologies pose several challenges for facilitating their use for hazard assessment, particularly from the regulatory submission standpoint. Sets of ‘big data’ must be condensed applying complex approaches and applying specific knowledge to obtain information that is pertinent for hazard assessment. Moreover, knowledge in this rapidly evolving area is not necessarily familiar to many researchers working in the regulatory settings. Accordingly, the lack of regulatory approval of omics technologies is not only linked to a lack of best practices frameworks and quality criteria, but also to a lack of confidence in the analysis and the level of uncertainty with respect to what constitutes enough data. Scientists and regulators have to first gain experience with the data obtained from this new technology and then to build confidence in its applicability.

ENVIRONMENTAL OMICS AND BIODIVERSITY

Generally, most of the environmental studies comprise culturing of isolated microorganisms or amplifying and sequencing conserved genes. However, some difficulties exist in understanding the complexity of large numbers of various microorganisms in an environment. This led to the development of new techniques allowing to enrich specific microorganisms for upstream analysis, such as single-cell isolation and analyses. Advanced tools in metagenomics and single-cell genomics (SCG) are paving the way to new methods of studying and understanding our environment.

Early environmental studies of organism diversity required samples cultivation to increase DNA quantity before genomic libraries to be constructed. At the time, sequencing of 16S and 18S rRNA was



2019-1-BG01-KA203-062371

the best option available to define environmental diversity. However, it became clear that majority of sample communities represents uncultivable species. Fortunately, polymerase chain reaction (PCR) of rRNA allows access to both cultivable and uncultivable sample diversity. Because PCR can be performed directly on environmental samples without cloning, it can be utilized to amplify targeted genes directly from the environment.

Importance of sequencing both noncoding rRNA and protein coding genes is growing, with specific interest in reconstructing bacterial genomes. A comprehensive sampling of all genes from all organisms existing in a complex sample is achievable via shotgun metagenomic sequencing. Using this method make possible to evaluate bacterial diversity and to detect the abundance of microorganisms in a specific environment. Moreover, it allows the reconstitution of complete genomes of uncultivable microorganisms. In addition to the discovery of new archaeal, bacterial, protozoan, algal, fungal, and eukaryotic species, this technique is crucial for the reconstruction of viral genomes. This was a critical advancement taking into account that viruses lack a shared universal phylogenetic marker such as bacterial 16S rRNA or eukaryotic 18S rRNA.

Another approach is the metatranscriptome approach which rather than using genomic DNA (gDNA) involves the collection of the entire RNA community. The latest is used to construct cDNA libraries that are sequenced. Although RNA is less stable than DNA, it gives a snapshot of the current state of the community by revealing up and down regulated genes. To date, one of the most important metatranscriptome studies are performed for the marine water communities.

1. Single-cell genomics

Metagenomic sequencing is a helpful tool for understanding environmental communities. However, assembling gene catalogs and composite genomes can be challenging. Furthermore, it is difficult to distinguish between genes that originate from the same or different organisms in genomes that are already assembled (Fig. 3). Therefore, there is considerable interest in developing a system to understand the organization of discovered genes and pathways within genomes.

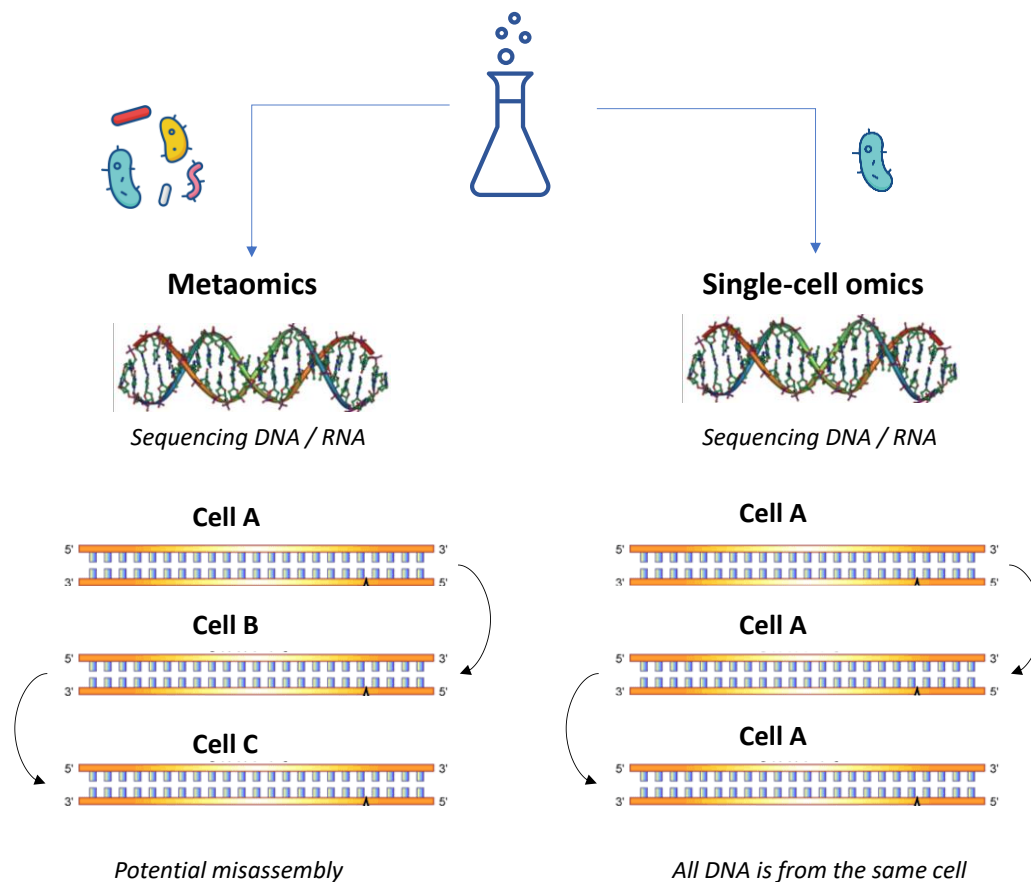


Fig.3. Metagenomics vs single cell genomics

The challenge of sample heterogeneity could be partially solved via using two techniques - scaling-up and deep-sequencing. Different approaches are required to compare metagenomic samples collected from different environments and to reveal the composition and organization of microorganism genomes in the environment. One of the approaches is to enrich the culture of a specific organisms based on their specific environmental functions. This type of targeted-metagenomic approach permits for the development of composite microbial genomes. Additionally, to the uncultivability of many organisms, other problems occur when faster growing microorganisms outgrow slower ones, subsequently introducing additional biases.

Hence, instead of culturing, a complex sample can be enriched for a target cell population using fluorescence-activated cell sorting (FACS). Using this approach helps sort cells on the basis of their shape, size, and density. Alternatively, specific organisms of interest can be enriched by handling a small



number of cells that possess specific function in the environment. With the development of whole genome amplification (WGA) techniques (e.g., multiple displacement amplification (MDA) or rolling circle amplification (RCA), the gDNA of a restricted number of cells can be amplified and sequenced. However, WGA techniques create potential deviations because the abundance of specific sequences vary and therefore are not equally amplified. Hence, gDNA amplification of defined populations with a restricted number of cells compromises the quantitative analysis of metagenomics.

Latest advancements in single-genome amplification techniques enable SCG using physical cell separation (usually by FACS on 96-well plates), cell lysis, and WGA. An amplified genome from a single cell can then be sequenced. While metagenomics is accomplished by collecting the microorganism community and extracting and sequencing total DNA, SCG separates and studies individual cells from the microorganism community. Obtained sequencing data provides quantitative information on genomic variability in microorganism populations. Gene insertions, deletions, duplications, and genome rearrangements can be studied on a single-cell level. In this way the complex metabolic pathways can be analyzed for an individual cell.

SINGLE-CELL ISOLATION AND PROCESSING

Studying the genome and its regulation at the population level is done by analyzing millions of cells at once. However, such investigations do not allow for a view of the heterogeneity in biological systems, nor do they characterize the current state of the population. That is why the SCG is gaining more and more popularity for studying both cultivable and especially uncultivable microorganisms. The process to obtain, multiply, and sequence genetic material from either live or dead cells is relatively simple. First the cell needs to be isolated and lysed and then the released genetic material is amplified and the genomic library can be constructed.

One of the approaches for single cell isolation is the micromanipulation. Cells of interest are identified, isolated, and examined microscopically. Suitable cells are isolated using a micropipette, laser source, optical tweezers, or by real-time microfluidic flow. Using microfluidics, a desired phenotype correlating to a specific genome can be selected. In addition, cells can be easily observed before capture.

Other approach is the random encapsulation. Cells are randomly selected by serial dilution. The diluted sample can then be transferred into microwells, or the cells can be encapsulated by microdroplets. Using this technique, individual cells can be separated, processed, and subjected to genomic analysis.

Flow cytometry is the most popular procedure of random encapsulation. FACS allows the isolation of cells possessing specific criteria, such as the size, shape, color, or even presence of specific nucleic acids or activities, using fluorescent dyes (Fig. 4). One of the main advantages of FACS is its high throughput, high sorting speed, and ability to distinguish live cells. Moreover, FACS can detect the

presence of cells in a droplet, reject empty droplets, and transferred the cells with desired properties into multiwell / microtiter plates. Then cells can be lysed, and genomes can be amplified in single wells.

Other possibility for single cell isolation is the microdroplet technology (Fig. 4). Microdroplets are the emulsion formed when cells in the water phase are vigorously mixed with oil. Oil droplets encapsulate cells in the water phase. Originally, PCR performed on an emulsion was thought to be much more specific than analysis of cells in the water phase alone. It was suggested that the yields of reaction products are greater and fewer chimeric byproducts are formed. Moreover, the droplets formed enclosed a limited number of template molecules (ideally only one). Latest developments in microfluidics permit to control the droplet size (nanoliter or even the picoliter), providing aqueous uniform (monodisperse) droplets in oil. One of the main advantages of the two-phase system of microdroplets over FACS is the possibility to handle single cells as separated units. Each droplet act as an independent well where cells can be grown and later screened for the products expressed. Released gDNA can be effectively amplified in microdroplets and used for sequencing-library preparation. Bacterial, yeast, plant, insect, and mammalian cells can be easily studied using microdroplet technology on agarose or other microgels. Even multicellular organisms can be encapsulated and grown in a droplet. Hypothetically, it is possible to measure any secreted molecule for which a fluorescently labeled ligand exists. Furthermore, the activities of various cell proteins can be monitored.

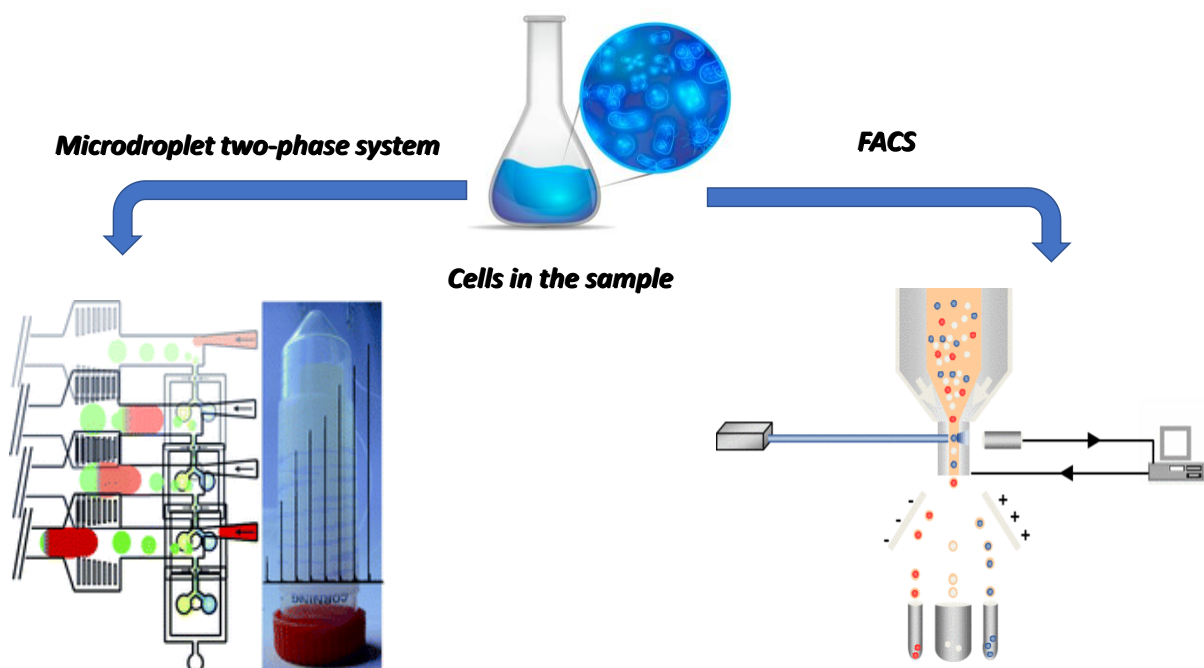


Fig. 4. Single cell isolation techniques



APPROACHES FOR STUDYING VARIOUS CELLS

Living cells can generally be classified into bacteria, archaea, and eukaryotes. Although viruses are not considered “living,” they constitute an important part of life science studies. Various single-cell analysis exists that are suitable for different organisms.

Viruses are present in practically every environment. They are the most abundant and diverse biological entities. However, to isolate single virus and sequence its genome, a suitable cultivable virus-host system must first be established. For example, eukaryotic algae are a host to an incredible diversity of viruses. However, using both FACS and single-droplet systems, viruses can theoretically be isolated without dependence on the viral-host system.

Archaea and bacteria have a similar cell structure. Only differences in cell composition and organization set these two domains apart. Bacterial cells are ordinarily 0.5 - 5.0 μm in diameter, while archaea can be larger and reach 15 μm in diameter. Basically, all methods described above can be used to separate and process single archaeal and bacterial cells. However, during FACS high currents may slow the growth of these organisms.

Eukaryotes differentiate from other life domains by their membrane bound nucleus and membrane-bound organelles. They may be multicellular or single-celled organisms. Cell size typically range from about 1 - 100 μm or they are ten-times larger than bacteria. Dilution, FACS, and droplet sorting are all appropriate methods for isolation of eukaryotic cells.

Multicellular organisms can theoretically also be isolated and processed by various methods. FACS for example can easily sort multicellular organisms. If incubated in droplets for a sufficient amount of time, single-celled organisms may synthesize a protein or ligand and have the opportunity to multiply, potentially creating a multicellular structure.

Molecular studies of our environment and ecology gain more and more knowledge for living communities. With the development of molecular tools, especially sequencing machines, it became possible to sequence whole communities from selected environments. Recent advancements in single-cell technologies facilitate the isolation and amplification of genomic material from single cells and allow structuring of numerous sequence-based libraries. Furthermore, data obtained from SCG supplements data obtained by metagenomics.



OMICS APPROACHES IN INDUSTRIAL BIOTECHNOLOGY AND BIOPROCESS ENGINEERING

Biotechnology utilizes biological processes, organisms, or systems to generate products and technologies that are improving human lives. The use of biological systems to produce bioproducts of commercial importance is a key component of the biotechnology industry. This biotechnological approach has found application in different areas: energy, material, pharmaceutical, food, agriculture, and cosmetic industries. The bioproducts made from biomanufacturing processes are usually metabolites and proteins, which are obtained from cells, tissues, and organs. The biological systems synthesizing these bioproducts can be natural or modified by genetic engineering, metabolic engineering, synthetic biology, and protein engineering. “Omics” technologies have great importance for biotechnology and metabolic engineering helping in characterizing and understanding metabolic networks. The significant amount of knowledge acquired from omics-driven experiments can be applied in the development of biotechnological tools and the advancement of metabolic engineering. This enables the manipulation of complex biological systems toward creating robust industrial biomanufacturing strategies.

1. Omics-Guided Biotechnology

“Omics” tools are used more and more in the development of biotechnological processes and the production of many vital products. Application of “omics” technologies in characterizing and understanding biological systems has permitted to select and predict phenotypes, which facilitates the optimization of biotechnological processes toward enhanced production (in quality and quantity) of commercially relevant products (Figure 5).

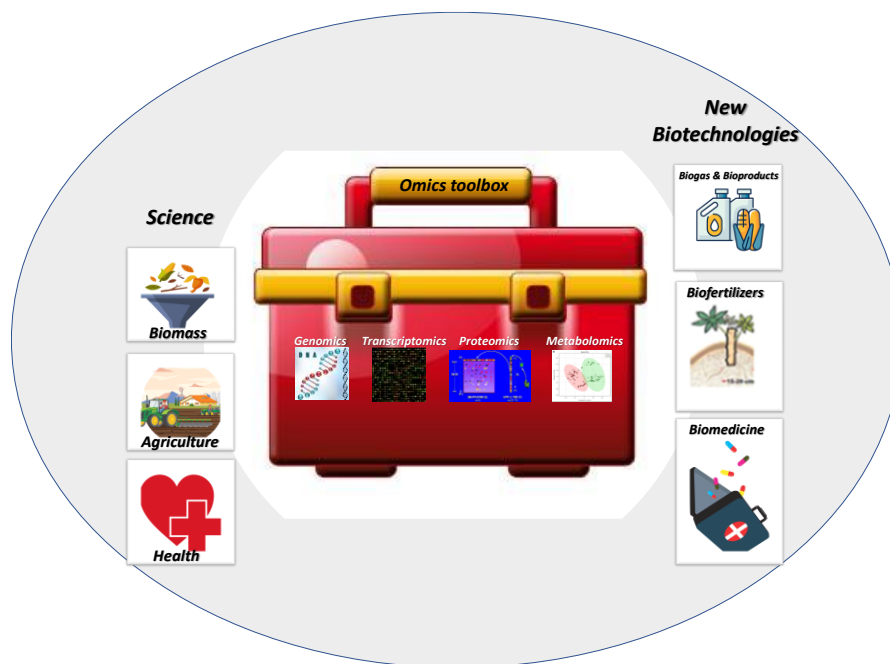


Fig. 5. Omics tools in Biotechnology

PRODUCTION OF BIOFUELS AND BIOPRODUCTS

Microbial production of natural compounds represents an attractive and more sustainable alternative to traditional petrochemicals. Its implementation has led to a growing catalog of natural products and high-value chemicals. For instant, the use of lignocellulosic biomass represents an economical approach to generate biofuels and bioproducts. However, to achieve stable conversion of low-cost raw material into value-added products at industrial levels needs systematic engineering plans. The Design-Build-Test-Learn (DBTL) cycle is becoming an increasingly implemented approach for metabolic engineering experiments. It represents a systematic and effective tool to force development efforts in biofuels and bio-based products. The DBTL cycle uses *in silico* approach to Design and Build genetic constructs in microbial hosts. Next, the information obtained from “omics” technologies, during the Test phase of the cycle, is transferred on to Learning processes (Figure 6). What is Learned is then fed back to new design cycles to achieve further strain development and optimization. Thus, a rapid optimization of microbial strains producers of any chemical compound of interest is facilitating. The weakest point in the DBTL cycle workflow is the Learning process since mathematical models are only as good as their assumptions. Therefore, both high quality and large “omics” data sets are required to improve training models, ensuring increased accuracy and reliability of the Learning process.

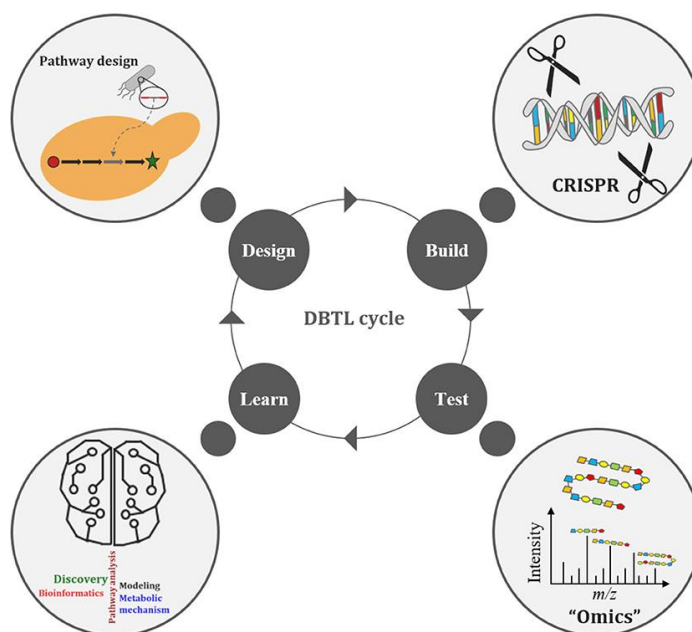


Fig. 6. DBTL cycle for optimization of biofuels and bio-based products

Often in the DBTL cycle genomic sequence information is the traditional approach utilized in the initial stages of a study. For example, barcode sequencing (Bar-seq) (a method using a short section of DNA from a specific gene or genes) can be used to study population dynamics of *Saccharomyces cerevisiae* deletion libraries during bioreactor cultivation, allowing the identification of factors that impact the diversity of a mutant pool. Single cell sequencing guided approach can be used to identify key mutated promoters for adjusting expression levels, thereby facilitating the dynamic regulation of microbial growth. Proteomics-guided approaches have been employed to engineer polyketide biosynthesis platforms for *in vitro* production of adipic acid in yeast. Additionally, metabolomics permits the assessment of pathway flux, carbon source utilization, and cofactor imbalance, which all contribute to the identification of pathway bottlenecks. Such metabolomics guided approach is already used for the characterization of the cannabinoid production in engineered *S. cerevisiae*. Cannabinoid analogs have been identified produced by several promiscuous pathway genes. Furthermore, application of metabolomics helped the design and optimization of a novel isopentenyl diphosphate-bypass mevalonate pathway in *E. coli* for C5 alcohol production. Recently, some authors have utilized the DBTL cycle to engineer *Rhodospiridium toruloides*, an oleaginous yeast species growing on lignocellulosic materials and producing the diterpene ent-kaurene, a potential therapeutic, exhibiting antimicrobial, anti-inflammatory, cardiovascular, diuretic, anti-HIV, and cytotoxic effects.



2019-1-BG01-KA203-062371

AGRICULTURAL AND FOOD BIOTECHNOLOGY

Recent innovations in agricultural biotechnology have led to new plant varieties, engineered by recombinant DNA technology and better responding to market demands and environmental challenges. In fact, “omics” tools in agricultural biotechnology have been used to enhance desirable phenotypic characteristics (e.g., color, taste, drought tolerance, pesticide resistance, etc.). “Omics” plays role not only in improving crop quality, consistency, and productivity, but also in the development of food crops with enhanced nutritional composition. Moreover, omics-driven systems biology helps in understanding the interactions between the “OMEs” and provide links between genes and specific characteristics.

In arable land soil is more susceptible to loss of structure, organic matter, minerals, and erosion. Thus, efforts are being made, via agricultural biotechnology, to provide a constant supply of nutrients essential to the growth of crops. An integral part of this approach is the use of biofertilizers. These are preparations containing specialized microbial inoculants that can fix, mobilize, solubilize, or decompose nutrient sources. Biofertilizers are generally applied through seed or soil and enhance nutrient uptake by plants. Widespread application of this approach, however, has been hampered by fluctuating responses of microbial inoculants across fields and crops. As a result, there is a pressing need to better understand the mechanisms underlying the interdependencies between soil microbial communities and host plant productivity. Recent genomics and exo-metabolomics studies showed that specific rhizosphere bacteria have a natural preference for certain aromatic organic acids exuded by plants. This fact suggested that plant exudation characteristics and microbial substrate uptake traits interact and form specific patterns of microbial community assembly. Furthermore, the application of genomics and transcriptomics to the study of phosphate uptake by various microalgae revealed a range of Pi transporters having specific expression patterns in relation to the availability of P. Currently, “omics” approaches are being used to study complex rhizospheric intercommunications, which is crucial to the development of new biofertilizers, thus promoting stable plant growth, better crop productivity and yield.

In the related field of food biotechnology, application of transcriptomics and metabolomics demonstrated that *Bacillus pumilus* LZP02 promote the growth of rice roots by enhancing carbohydrate metabolism and phenylpropanoid biosynthesis. Further, the application of “omics” in starch bioengineering provides better understanding on the most important enzymes for its biosynthesis. This facilitates the prediction on how starch-related phenotypes can be modified and ensures further progress in the research field of rice starch biotechnology. “Omics” also help solving issues related to food quality and traceability, to protect the origin of food, and discover biomarkers of potential food safety problems. The advance investigation of wine microbiome has a great influence for wine industry helping in better understanding of factors transforming grapes to wine, including flavor and aroma. “Omics” characterization of the complex relationships between these microorganisms, the substrate and environment, is essential to shaping wine production. Finally, combining “omics” technologies with genome editing of food microorganisms can be exploited to generate improved probiotic strains, develop innovative bio-therapeutics and alter microbial community structure in food matrices.



“OMICS” TECHNOLOGIES AND COVID-19

The coronavirus disease 2019 (COVID-19) is characterized by the Severe acute respiratory syndrome coronavirus 2 [i.e., SARS-CoV-2, which binds to the ACE2 receptor in the lung and other organs]. Due to its worldwide spread a global pandemic has been announced and much of the world's economy has been slowed. Up to April 2021 there are more than 138 million confirmed cases and 2.5 million confirmed deaths globally (<https://www.worldometers.info/coronavirus/>). Thus, there is an urgent need for an effective countermeasure to mitigate the spread of the pandemic.

Therefore, efforts are ongoing to fast-track the development and production of safe and effective vaccines against SARS-CoV-2. Preceding knowledge of SARS and Middle East respiratory syndrome (MERS) has facilitated targeting the spike protein as the viral antigen (via the ACE2 receptor). Furthermore, the genome sequencing of the SARS-CoV-2 in January 2020 made it possible to accelerate the development of next generation mRNA and vaccine platforms that encode for the antigen. Once injected into a host, the mRNA, encapsulated into lipid nano-particles, remains in the cytoplasm. When DNA is used, encased in an attenuated adenovirus vector, it enters the nucleus. The host cell translates these genetic materials into the spike protein. It settles on the surface of the cell and provokes an adaptive immune response mediated by T cells (e.g., CD4+ and CD8+) and B cells (i.e., antibodies). These vaccines were reported to be effective against SARS-CoV-2 in recent clinical trials, which underlines the significance of genomics to this new era of vaccine development.

Since the beginning of the outbreak of SARS-CoV-2 a protein interaction was created map and using a proteomics-based approach targets for drug repurposing was revealed. By applying proteomics analysis 26 of the 29 SARS-CoV-2 proteins were cloned, affinity tagged and expressed in human cells and the associated proteins were identified. A total of 66 human proteins or host factors were discovered as potential drug targets of 69 compounds. Two sets of these pharmacological agents showed antiviral activity. Moreover, computational immunoproteomics studies were performed supporting the lab-based investigations and allowing the evaluation of diagnostic products specificity, the forecast of potential vaccines adverse effects and the reduction of animal models utilization.

Furthermore, recently a method was developed based on metabolomics that is able to distinguish COVID-19 patients from healthy controls via the analysis of 10 plasma metabolites. Additionally, the data from lipidomics study suggests that monosialodihexosyl ganglioside enriched exosomes could be involved in pathological processes. Proteomics and metabolomics investigations in COVID-19 patient sera revealed that SARS-CoV-2 infection causes metabolic dysregulation of macrophage and lipid metabolism, platelet degranulation, complement system pathways, and massive metabolic suppression. The analysis of plasma metabolomic signatures showed similar profile to those described for sepsis syndrome. Furthermore, transcriptomics results showed upregulation of genes related to oxidative phosphorylation both in peripheral mononuclear leukocytes and bronchoalveolar lavage fluid. All this information suggests a critical role of mitochondrial activity during SARS-CoV-2 infection. Understanding the clinical presentation of COVID-19 as well as metabolomic, proteomic, and genetic profiles could help in discovering specific diagnostic, prognostic, and predictive biomarkers, ensuring



2019-1-BG01-KA203-062371

the development of more successful medical therapy. Moreover, differentiating metabolic biomarkers of severe vs. mild disease states in the lung during respiratory infections could lead to the discovery of novel therapeutics that modulate symptom and disease severity.

NUTRI-OMICS RESEARCH

More than a decade has passed since the idea of nutrigenomics was first introduced. Nutrigenomics, also referred to as nutritional genomics, nutritional omics, or nutri-omics, can be defined as an area of food and nutrition research making use of profound analyses of molecules or other physical phenomena.

Considering the complexity of the human body and its various interactions with food, it is conceivable that holistic analyses of food- body interactions are an important prerequisite for better understanding the effect of dietary components. The main assumption is that the combination of different omics platforms will provide a deeper insight into the influence of food components and the mechanism of their actions.

1. Genomics and nutrition

Human genes have different variants in the population. Having in mind that response to nutrition is a multigenic process, it is not surprising that non-related individuals may respond differently. The key hypothesis proposed in recent years stated that genetic variation underlies variation in nutrition-related disorders and risk for disease. In fact, nutrigenetics tries to explain how and to what extent nutrition-related traits and disorders are influenced by genetic variation. In other words, the key questions of nutrigenetics are:

- Which genes are involved in determining a defined characteristic?
- What is the functional identity of the variation by which people differ for this characteristic?
- How can this knowledge be used for the benefit of the population?

Usually, researchers define 'candidate genes' for a characteristic or disorder and search in those genes for variation. This is described as the 'hypothesis-driven' approach because the selection of the genes is based on their presumed function. For example, the genes for lipoproteins are used as candidates for obesity and the genes for insulin signaling - as candidates for diabetes. Other attractive candidates in humans are the orthologous genes underlying animal models with a Mendelian segregation of a characteristic. A well-known example is the db/db mouse. It is a model for diabetes in which the gene



2019-1-BG01-KA203-062371

for the leptin receptor was identified. The human homologue was then tested in persons with an impaired glucose tolerance. One variant (allele) of the gene was identified and was proven that it was associated with a significantly higher frequency of diabetes occurrence in comparison to the general population.

Other methods applied is related with studying the preferred segregation of an allele from parents to an affected child (transmission disequilibrium test) or to more affected siblings (sib-pair analysis). If a gene is found to be linked to the characteristic, it remains to be proven whether the associated or linked allele itself is the risk factor, or whether it could be used only as a marker for a nearby causative variation. If the genetic variation is linked with amino acid variation in the protein, a functional test could be developed and the allelic proteins can be compared for their enzymatic activity, DNA-binding affinity, etc.

Another approach to search for risk-conferring genes for a certain characteristic or disorder is the total genome scan. Hundreds of polymorphisms are detected with a random distribution across the genome but with a known chromosomal location. For every polymorphic site it is then identified whether an association exists between the allele and the characteristic. Using this approach risk loci can be identified for many human nutrition-related disorders like obesity and diabetes.

After the second world war in many Western countries, it was observed that the incidences of spina bifida gradually decreased. It was suggested that the improvement of the diet had brought forth this effect. A large epidemiologic study was started. The results obtained showed that the enrichment of the diet with vitamins was able to prevent the occurrence and recurrence of spina bifida in humans with more than 50%. Folic acid was discovered to be the active substance and now in many countries pre-conceptional folic acid supplementation is recommended as a general preventive measure. Simultaneously, it has become clear that concerning the risk for a child with spina bifida, the women in the population can be roughly divided into three groups: [i] not at risk even at a low folate intake, [b] at risk at low dietary folate but can be helped by increased folate intake, and [c] at risk despite extra folate intake. Genetic predisposition was presumed, and candidate genes taken from the folate metabolism were examined for genetic variation. This led to the detection of the 667C->T and 1298A->C alleles in the gene for methylenetetrahydrofolate reductase (MTHFR) as risk factors. However, these risk alleles are discovered to some extent in all three groups of women illustrating that genetic testing in the context of a personalized diet would be inadequate. Moreover, it became clear that not all the relative risk could be explained by these alleles. Therefore, the search for genetic variation in other genes is continued and new candidate genes may be found.

Currently, several hundreds of genes related to specific nutrition-related characteristics and disorders have already been identification via using genetic experiments in various populations and the investigation of animal and *in vitro* model systems. Finally, the nutrigenomics and nutrigenetics research should lead to the comprehensive understanding of the etiology of those characteristics and disorders with respect to the interacting genes, the dietary components, and the relative risk conveyed by both genetic variation and diet. This combined knowledge will permit the genetic profiling of every individual and thereby assess her/his risk for developing nutrition-related disorders. Based on this personal risk



2019-1-BG01-KA203-062371

profile of genetic factors, a ‘personalized diet’ could then be proposed by which the onset of a disorder could be prevented or at least delayed. Already some companies offer genetic testing for alleles that were found to be associated with certain characteristics. For example, the ApoE allele is tested as a risk factor for cardiovascular disorders and an allele of the alcohol dehydrogenase gene is used to predict potential alcohol sensitivity and (ab)use. However, it is important to note that the presence of certain allele may be said to increase the risk for a disorder by 100%, while in absolute terms the risk would usually still be very small like an increase from 0.001 to 0.002. Additionally, testing only one genetic factor might give an incomplete or even a wrong view of the situation. Nowadays, we have no idea of all the genetic factors and how they interact. Till more information is gathered, the advice based on simple testing probably has to remain simple, sounding like “drink less alcohol” or “eat less saturated fat”.

2. Transcriptomics and nutrition

Transcriptomics is the most widely employed in food research because of the many advantages of the DNA microarray technology, including the comprehensiveness of the expression data, established protocols, and high reliability and reproducibility of the data. It has been also reported the alternations of global gene expression in response to different dietary changes such as nutritional deficiency, fasting, overeating, and ingestion of specific food factors. As one example of the performed attempts to acquire reference data a transcriptome analysis of the liver of rats treated with mild caloric restriction has been performed. When using animal experiments, it was shown that a modest reduction of food intake or altered intake pattern could be sufficient for occurrence of significant metabolic changes. Therefore, it is important to distinguish between the direct effects of the food component and the secondary effects caused by the change in eating behavior. When rats were fed a diet with 5 to 30% less food than that consumed by an ad libitum-fed group for 1 week or 1 month it has been observed restriction – dependent changes in the expression level of cyp4a14. In fact, the cyp4a14 gene was induced by even a low level of caloric restriction. This data suggests that the gene can be used as a biomarker for the beneficial effects of food on energy metabolism.

3. Proteomics and nutrition

Many food and nutrition research studies have been carried out by using proteomics approaches. For instant, the effect of mild caloric restriction described above was also examined by proteomics. Nine significantly up-regulated proteins and nine down-regulated proteins have been detected after the proteome comparison of the liver of rats treated with 30% food restriction with control ones. Ten percent restriction caused the up-regulation of 9 proteins and the down-regulation of 2 proteins. An interesting discovery was the up-regulation of prohibitin, whose involvement in the regulation of longevity was recently revealed. This result suggests that prohibitin can be applied as an effective biomarker of the beneficial effects of food factors. Although the current stage of proteomics research is much less



2019-1-BG01-KA203-062371

extensive compared with that of transcriptomics, the study described here together with other nutritional proteomics research findings indicates that proteomics is a highly promising tool for the discovery of biomarkers.

4. Metabolomics and nutrition

About ten thousand different types of major metabolites exist in animal bodies, while the number of proteins is thought to exceed 100,000. This feature of metabolites is expected to result in more comprehensive features of metabolomics analysis than proteomics ones. However, the analysis of metabolites is in fact fraught with difficulties and usually requires the use of sophisticated techniques and high skill level personnel. Another difficulty originates from the width of the metabolite's abundance. Regardless of these obstacles, metabolomics is a powerful tool in food and nutrition science.

Many investigations showed that gut and serum metabolism is influenced by the change in gut microbiome composition. The gut microbiome and its alternation in relation to the diet are essential when evaluating dietary intervention trials with metabolomic end effects. The comparison between germ-free mice colonized by human baby flora and conventional mice showed the complexity of diet modifiable microbiome/metabolome covariation. In this study, the effect of the intestinal microbiome on plasma metabolites was studied. Data revealed that more than 10% of the plasma metabolome is directly dependent upon the microbiome. Some examples for microbial dependent compounds in plasma include cinammic acid, glycine conjugated compounds, hippuric acid, and other plasma metabolites. The gut microbiome also directly affects the host's ability to metabolize lipids, carbohydrates, and proteins, and can carry out several phase II detoxification reactions. There is also evidence that gut microorganisms utilize nonnutritive phytochemicals. For example, several investigations demonstrated that levels of three microbiome-dependent diet-derived metabolites, choline, trimethylamine N-oxide, and betaine, could predict risk for cardiovascular disease in mice.

5. Nutrition and other OMICs

Many other omics platforms are also a focus of nutriomics research. It was shown that epigenetic alteration could be caused by the nutrition diet during fetal development and could affect the predisposition to lifestyle-related diseases in later life. For example, children of mothers who suffered over- or undernutrition during pregnancy have increased risk of developing obesity, diabetes, hypertension, cardiovascular diseases, etc. Many studies have proven the involvement of epigenetic modifications in such acquired susceptibility. Another promising target of omics approaches in food science is associated with the RNA transcripts that do not encode proteins. MicroRNA (miRNA) is a subtype of these non-coding RNAs. Precursors of miRNAs (pri-miRNA) are long products which are cleaved to yield mature miRNAs of 22 nucleotides in length. Mature miRNAs regulate the levels of



mRNA degradation, mRNA translation, and even gene transcription. In the case of human cells, about 1,000 miRNAs are identified and are supposed to regulate the expression of more than half of the protein-coding genes. Considering the accumulating data supporting the key role of miRNA in the development of diseases and the maintenance of health, the information on the status of miRNAs is no doubt vital for the understanding of the interaction between food components and the body. Global miRNA analysis now can be easily performed by the use of commercial miRNA arrays.

With the introduction of new technologies and acquired knowledge, the number of fields in omics and their applications in various areas are rapidly increasing in the postgenomics era. Such emerging fields - including pharmacogenomics, toxicogenomics, regulomics, spliceomics, metagenomics, and environomics - present promising solutions to combat global challenges in biomedicine, agriculture, and the environment.

References

- Amer B, Baidoo EEK. 2021. Omics-Driven Biotechnology for Industrial Applications. *Frontiers Bioeng. Biotech.*, 9, Art. 613307: 1-19.
- Azizipour N, Avazpour R, Rosenzweig DH, Sawan M, Ajji A. 2020. Evolution of Biochip Technology: A Review from Lab-on-a-Chip to Organ-on-a-Chip. *Micromachines*, 11: 599.
- Canzler S, · Schor J, · Busch W, et al. · 2020. Prospects and challenges of multi-omics data integration in toxicology. *Archives of Toxicology*, 94:371–388.
- Horgan RP, Kenny LC. 2011. ‘Omic’ technologies: genomics, transcriptomics, proteomics and metabolomics. *The Obstetrician & Gynaecologist*, 13:189–195.
- Joseph P. 2017. Transcriptomics in toxicology. *Food Chem Toxicol.*, 109(1): 650–662.
- Karahalil B. 2016. Overview of Systems Biology and Omics Technologies. *Curr. Med. Chem.*, 23, 1-10.
- Karczewski KJ, Snyder MP. 2018. Integrative omics for health and disease. *Nat Rev Genet.*, 19(5): 299–310.



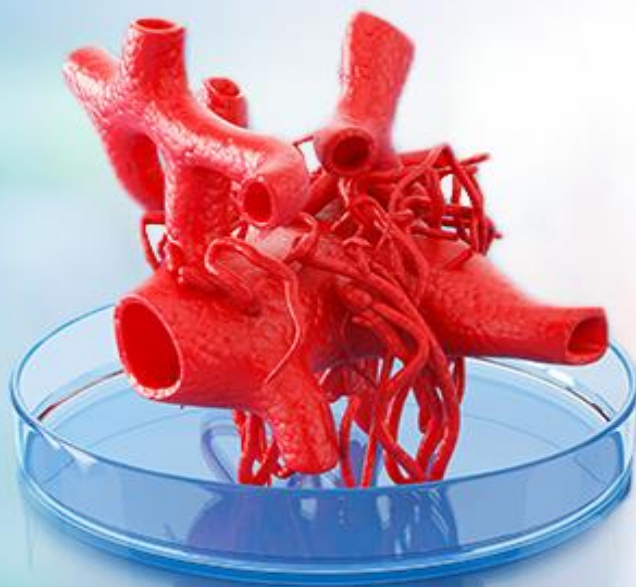
2019-1-BG01-KA203-062371

- Kato H, Takahashi S, Saito K. 2011. Omics and Integrated Omics for the Promotion of Food and Nutrition Science. *J. Trad. Complement. Medicine*, 1 (1): 25-30.
- Kim DH, Kim YS, Son NI, Kang CK, Kim AR. 2017. Recent omics technologies and their emerging applications for personalized medicine. *IET Syst. Biol.*, 11 (3): 87-98.
- Kodzius R, Gojobori T. 2016. Single-cell technologies in environmental omics. *Gene* 576: 701–707.
- Mariman ECM. 2006. Nutrigenomics and nutrigenetics: the 'omics' revolution in nutritional science. *Biotechnol Appl Biochem.*, 44(3):119-28.
- Mousumi D, Prasad GBKS, Bisen PS. 2010. Omics technology. In: *Molecular Diagnostics: Promises and Possibilities*, Dordrech Heidelberg London, Springer, pp 11-31.
- Ryan EP, Heuberger AL, Broeckling CD, Borresen EC, Tillotson C, Prenni JE. 2013. Advances in Nutritional Metabolomics. *Current Metabolomics*, 1: 109-120.
- Sauer UG, Deferme L, Gribaldo L, et al. 2017. The challenge of the application of 'omics technologies in chemicals risk assessment: Background and outlook. *Regulatory Toxicology and Pharmacology*, 91: S14 - S26
- Simões T, Novais SC, Natal-da-Luz T, et al. 2018. An integrative omics approach to unravel toxicity mechanisms of environmental chemicals: effects of a formulated herbicide. *Scientific Reports* 8:11376
- Titz B, Elamin A, Martin F, et al. 2014. Proteomics for systems toxicology. *Computational and Structural Biotechnology Journal*, 11: 73–90.
- Vlaanderen J, Moore LE, Smith MT, et al. 2010. Application of Omics Technologies in Occupational and Environmental Health Research; Current Status and Projections. *Occup Environ Med.*, 67(2): 136–143.
- Yilmaz B, Yilmaz F. 2018. Lab-on-a-Chip Technology and Its Applications. In: *Omics Technologies and Bio-engineering: Towards Improving Quality of Life*, pp. 145, Elsevier Inc.



Project website: www.digit-biotech.eu

The European Commission's support for the production of this publication does not constitute an endorsement of the contents, which reflect the views only of the authors, and the Commission cannot be held responsible for any use which may be made of the information contained therein.



LO2 THE APPLICATION OF 3D PRINTING AND ICT FOR GREEN PRODUCTS AND PROCESSES

Basic level

AUTHOR:

TRAYANA NEDEVA



Contents

3D printing principles of operation	3
Defininitions.....	3
Brief history of the field.....	4
Advantages and disadvantages of 3D printing as a method of manufacture.....	5
Basics of 3D printing processes and materials.....	8
Types of 3D printing processes.....	8
Post-processing requirements for different 3D printing technologies.....	16
Which process for which? Basic considerations when selecting 3D printing technology suited for a particular application.....	17
Commonly used software for 3D printing.....	19
3D printing design guidelines	20
References	23

3D printing principles of operation

DEFININIONS

3D printing creates a tangible object by adding successive layers representing cross-sections of the object. The simplest allusion there is making a layered cake (Fig. 1), only the layers are numerous and typically very thin, only about 1/5 of the millimetre.



A.

B.

Figure 1. Layers and layering in 3D printing.

A – Layered cake, representing the basic principle of 3D printing. Source: Caroline's Cakes - Own work, CC BY-SA 3.0,

<https://commons.wikimedia.org/w/index.php?curid=12799030>

B – 3D printed chocolate castle. Source: FM1418, CC BY-SA 4.0

<<https://creativecommons.org/licenses/by-sa/4.0/>>, via Wikimedia Commons

3D printing is close, both in process and material, to routine ink-and-paper 2D printing. Nevertheless, in typical 2D printing only an illusion of depth is created using perspective and colour or grayscale, whereas 3D printing creates tangible 3D objects. These may have a myriad of purposes, from rapid creation of prototypes for research and industry to printed objects for everyday or special use; machine parts; custom-made culinary items such as the chocolate castle presented in Fig. 1B; printed implantable materials for the regenerative medicine, and, potentially, 'replacement parts' for the human body; and novel forms of art.

A basic desktop 3D printer with PC is presented on Fig. 2.

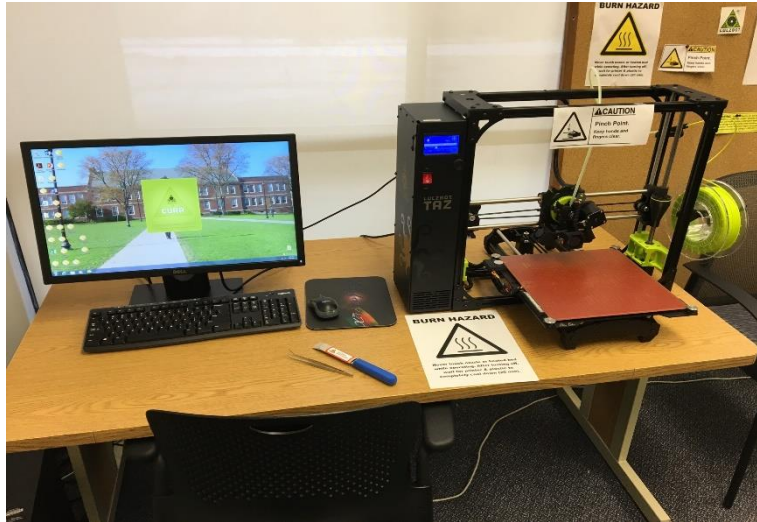


Figure 2. LulzBot TAZ (FAME 3D, Fargo, ND, USA), an open-source 3D printing machine based on FDM technology. Source: Ochs, J. - College, CC BY 4.0, <https://commons.wikimedia.org/w/index.php?curid=59898086>

Typically, the process of 3D printing of an object is additive (hence 3D printing is also known as “additive manufacturing”). It is achieved by depositing layers upon layers of a printable material (commonly termed as ‘3D ink’) until the object is complete.

3D printed objects need not to be solid within (though they might be). They may contain any type of cavities that has been set up in the data file used for printing, because every layer represents a cross-section at a specific level in the manufactured object.

A short time-lapse gif illustrating the basic process of 3D printing may be viewed here:

https://upload.wikimedia.org/wikipedia/commons/e/ed/Robot_3D_print_timelapse_on_RepRap_Pro_Fisher.gif

The most commonly used 3D printing nowadays is fused deposition modelling (FDM), although other types are rapidly gaining popularity.

BRIEF HISTORY OF THE FIELD

It is commonly believed (and cited) that the very idea of 3D printing did not exist until the 1950’s when the sci-fi writer Raymond F. Jones published a short story describing use of ‘molecular spray’ directed by information from a pre-recorded data tape. Nevertheless, the possibility for controlled hands-off creation of a complex tangible object following virtual directions was foreseen about 50 years earlier by the great visionary Joseph Rudyard Kipling in his children’s story ‘How the camel got its hump’. The first attempt of 3D printing, however, did not arrive until the 1970’s, and it did entail both creation



2019-1-BG01-KA203-062371

and removal of objects. Specifically, this was the patented ‘Liquid metal recorder’ by Johannes F. Gottwald (US3596285A) using electrically conductive metallic ink propelled under pressure against a surface. The melted ink would flow at printing temperature but will solidify as it contacts the surface, then it could be removed by increasing the temperature above the melting point, enabling the reuse of the ink and the surface for further use.

As with most novel concepts, the idea of 3D printing did not meet early success. In the early 1980’s, a patent was filed by Hideo Kodama in Japan for additive manufacturing of ‘stereoscopic figure drawing device’ using UV-hardening polymer where the areas of UV irradiation were controlled by pre-set masking pattern (JP S56144478 A). Despite prompt publication of his results, Kodama could not raise much interest in his project and it was subsequently cancelled.

Four years later in France the same fate met the concept for stereolithography presented by Le Méhauté, de Witte, and André and their subsequent patent for the process, purportedly "for lack of business perspective" [<http://www.primante3d.com/inventeur/>].

Around that time the American William E. Masters filed the first of his series of patents (namely, the patent for ‘Computer Automated Manufacturing Process and System’ (US 4665492) that eventually laid the foundations of 3D printing as we know it.

Only two years later the American Charles Hull was granted a patent for his own ‘apparatus for production of stereolithographs’ (US4575330A), founded his own company and eventually released the first ever commercial 3D printer, the SLA-1, in 1987. The patent for the most commonly used in present day type of 3D printing – the FDM - was filed in 1989 in the US by S. Scott Crump (US5340433A) and the first FDM equipment was released in 1992. Hull also gave probably the simplest and yet complete definition of 3D printing to date, namely: "*A system for generating three-dimensional objects by creating a cross-sectional pattern of the object to be formed...*", in the description of his patent for ‘Method for production of three-dimensional objects by stereolithography’ (US5174943A).

ADVANTAGES AND DISADVANTAGES OF 3D PRINTING AS A METHOD OF MANUFACTURE

There are numerous advantages to using 3D printing rather than traditional methods of manufacture. Some of these are listed below:

1. Complex design

Since the object is not cut out of a block, 3D printing may handle different designs with very complex structure.

2. Possibilities for last-minute modifications.

The design is easily modifiable and a variety of different prototypes of an object may be rapidly manufactured and tested.

3. Easy and rapid tailoring of design to suit individual needs



2019-1-BG01-KA203-062371

Unlike traditional methods of manufacturing, the introduction of needed modifications concerns the data files rather than the machine itself and may be carried out with very little notice.

4. Yields lightweight and durable objects

That depends on the material used, but most commercial 3D printers use plastic, although use of other materials such as plaster, metal, metallic alloys, etc. is also possible.

5. Relatively rapid production on smaller scales

Depending on size and complexity of the design, most objects may be manufactured within hours, although larger ones may take more time.

6. Low (compared to traditional methods of manufacture) waste of material

Again, since there is no cutting, drilling or boring, the wastage of material is negligibly low. An exception is powder-based 3D printing where excess powder is usually lost.

7. May be more cost-effective than traditional methods of manufacture

That depends largely on the material and the process used for manufacturing.

8. The ease of access of researchers, industry and the public to 3D printers is increasing all the time

At present, gaining access to a 3D printer is relatively easy, although there is typically a queue of customers waiting to use the equipment. The waiting time depends on the make and model of the printer, the institution it is been hosted by and the type of use the 3D printer is being put to.

9. May be more environmentally friendly than traditional methods of manufacture

Again, this depends of the type of material used, the intensity of use and the maintenance of the equipment. Discarded objects, wastewater and small particles emission, however, may pose a problem (see below).

10. Special uses of 3D printing in reparative and regenerative medicine (3D-bioprinting)

Significant research and development effort is currently invested in developing a new generation of implantable materials. Instead of simply providing inert support, friction-friendly surfaces and bridging between intact portions of damaged tissue (which are the main functions of most implantable materials of today), the new materials may also presumably become a part of the natural habitat of the cells of the damaged tissue. Such materials may be purely of artificial origin but resemble biological tissue (biomimicking materials). One potential use of 3D bioprinting is providing optimal 3D environment of implantable materials for colonisation with living cells from the surrounding tissue. Another very promising application of 3D bioprinting is the potential for creating living tissue (and, potentially, functioning replacement organs and various 'spare parts' for living bodies) using living cell-based materials as printable 'bioink' (see below).

Potential limitations of 3D printing compared to traditional methods of manufacture may be:

1. Limitations imposed by errors in design

While there are virtually no limits to potential design types, a single error in the design may lead to manufacture of unusable object/s. While some of the errors of design may be minimised by finishing, it may increase the time to process an order and may decrease the durability of the object.

2. Limitation imposed by material



2019-1-BG01-KA203-062371

Most 3D printers use as ink some type of plastic polymer, although some may also print with metal and metallic alloys. Objects that are produced and then discarded may pose a problem if the material is not biodegradable and/or recyclable. Printing and post-processing may result in emission of particles that contribute to environmental pollution and may have carcinogenic potential when breathed and ingested.

Issues concerning the material for bioprinting may be aggravated further in 3D bioprinting where viability of living cells and biocompatibility is of utmost importance.

3. Limitations imposed by size of the object

Very large objects may be difficult to manufacture in one piece. Different sections of the object may need to be printed separately and then assembled together. This may increase the time and costs for manufacturing and may decrease the durability of the object (see below).

4. Integrity of the produced objects may be compromised

This may be an issue especially when parts are produced separately and then assembled together. Under physical stress, the layers may come apart (especially in FDM-produced objects) and complex objects may be brittle and delicate to handle.

5. 3D-printed objects may need finishing

Essentially, this makes no difference with traditional manufacturing. Again, this may increase the time and costs for manufacturing.

6. Cost generally not reduced with larger batches

In traditional manufacture, when the equipment is working at full capacity, the costs of manufacture per object or per batch generally tend to be lower. This is not the case with 3D printing and large volumes of manufacture may be costly.

7. Copyright Issues

Quite self-explanatory. The issues entail not only the manufactured objects but also the software, hardware and processes used for manufacturing them.

8. Ethical issues, concerning mainly the 3D-bioprinting of living tissue

9. May indirectly cause reduction of jobs in the traditional manufacturing sector.



Basics of 3D printing processes and materials

TYPES OF 3D PRINTING PROCESSES

Many types of 3D printing technologies currently exist. What they all have in common is that tangible objects are created by deposition of successive layers rather than cutting out the object out of a solid block and/or drilling, milling or boring. Every consecutive layer represents a cross-section of the object under construction. The process is controlled by a computer equipped with designated software for creation of design files, file processing and object printing (see further for details).

Several of the most common technologies for 3D printing are briefly presented below. This list is by no means exclusive.

- Liquid or semisolid ink-based technologies: SLA, FDM, EXT, PolyJet

All these three are based on deposition of layers of liquid (more or less viscous) ink that are then fused to one another by heat or directed light beam (emitted by an UV lamp or laser). The ink may be extruded from a nozzle or, when it is of higher consistency, fed from a spool.

- Stereolithography (SLA)

Stereolithographic 3D printing is perhaps the most easily comprehended of all 3D printing technologies, probably because photopolymerisation is commonly used in modern dentistry. Basically, stereolithography is light-curing of photosensitive material extruded in a layer-by-layer fashion onto a movable platform. The wavelength of the light source is most often in the UV range but other wavelengths may also be used. SLA materials polymerise via free-radical polymerisation.

Different parts of the object may need to be supported in the making of the object so as to avoid the collapse of the whole structure. Successive layers are made to adhere to each other by cross-polymerisation of the just polymerised layer to the newly deposited layer where polymerisation is still incomplete. Since polymerisation may take some time to complete, the printed object usually needs some time in order to harden, then supports are removed (if any of these remain after the last layer has been deposited), then a final post-processing may take place.

A SLA printed 3D model is presented in Fig. 3.



2019-1-BG01-KA203-062371



Figure 3. Stereolithograph of bony labyrinth of the inner ear in man. Source: Didier Descouens, CC BY-SA 4.0 <<https://creativecommons.org/licenses/by-sa/4.0>>, via Wikimedia Commons

The ink used in SLA-based printing is usually liquid in its unpolymerised form. It is mostly epoxy or acrylic-based, although trimethylene carbonate, polycaprolactone, and polylactide-based inks may also be used. Use of such materials yields printed objects with significant rigidity that are, nevertheless, quite fragile. Thus, SLA is quite suitable for rapid prototyping and research purposes (e.g., tissue engineering scaffolds) but not for objects that need to be durable. SLA has quite high resolution (minimal layer thickness is 50 µm) and therefore is very reliable when fine detail is crucially important.

Objects created by SLA may be light-sensitive and may tend to disintegrate over time, especially when kept in light environments.

Solvents are rarely used in SLA printing, therefore, the risk of escape of fumes to the atmosphere is low. Due to the fragility of SLA-printed objects, nevertheless, discarded objects may present an environmental hazard. The use of UV calls for special precautions in order to avoid accidental exposure of surfaces, objects and operators to the curing beam. Some of the inks used for stereolithography may have significant neurotoxic and carcinogenic potential. Also, UV-mediated formation of ozone may need to be considered.

- Fused deposition modelling (FDM), also known as fused filament fabrication (FFF)

At present, this is the most commonly used technology for 3D printing. FDM technology is based on extrusion of a filament of thermoplastic ink fed from a spool through a moving heated printer nozzle. The ink within the nozzle is heated just above its melting point and is extruded in semi-solid form that solidifies almost instantly after a layer is deposited. The object is built upon a moving platform that is gradually lowered as the structure grows (Fig. 4).

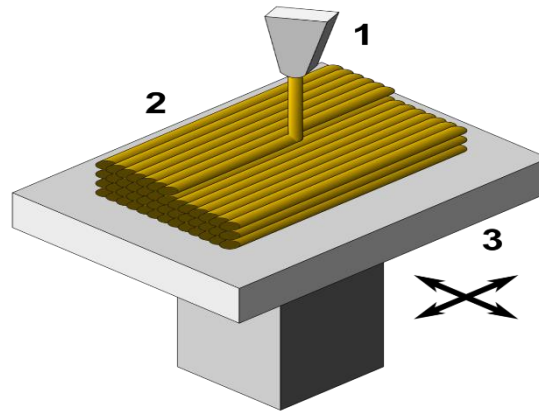


Figure 4. Working principle of FDM printers. Source: By Gringer - Own work, CC0, <https://commons.wikimedia.org/w/index.php?curid=73614647>

The minimal layer thickness for FDM is 100 μm . The most commonly used ink materials for FDM are acrylonitrile butadiene styrene (ABS), polylactide (PLA) and polyethylene terephthalate glycol (PETG). Others, such as polyphenyl ether (PPE), polyurethane (PU), polyethylene terephthalate glycol (PETG), polyphenylsulphone (PPSP), polycarbonate (PC), polyetherimide (PEI), polyether ketone (PEEK), polyamide (PA, commonly known as nylon) are also in use. Polyvinyl alcohol (PVA) may also be used, but since it is hydrophilic, PVA is most commonly used for manufacturing easily removable supports for a structure printed using other type of thermoplastic ink.

The heatable ink for FDM may be also in the form of rods or granules. A variation of FDM uses cold extrusion of unheated ink, usually supplied as slurry or paste.

A FDM printer with spool of filament is presented in Fig. 5.



2019-1-BG01-KA203-062371



Figure 5. PRUSA i3 MK2 FDM printer (Prusa Research, Prague, Czech Republic) with spool and a completed object. Image source: Wikimedia Commons, <http://reprap.org/wiki/File:Prusai3-MK2.jpg>.

Being relatively low-cost, FDM is especially popular among hobbyists and desktop model makers. In addition, FDM is commonly used for rapid prototyping in engineering, in manufacture of high-performance parts and machinery, in education and in art.

The objects manufactured by FDM are significantly more durable than these produced by SLA and with very good quality of detail reproduction. Different quality FDM inks yield objects with different properties with regard to durability, rigidity and flexibility (see below).

Disadvantages of 3D printing by FDM are mainly related to potential environmental hazards (use of solvents, emission of small particles during post-processing, and the fact that the discarded objects are quite durable).

- Semi-solid Extrusion (EXT) 3D Printing

EXT 3D printing is another technology based on extrusion. The ink is usually polymer-solvent mixture with the consistency of paste. EXT materials are not thermoplastic, allowing for printing at low temperatures. Again, the object is built upon a moveable platform.

EXT is commonly used in research (e.g., tissue scaffolds) and for pharmaceutical purposes (targeted and/or timed delivery of drugs). Biosafety is ensured by using inks based on

polyvinylpyrrolidone, polyethylene glycol, methycelullose, hydroxypropyl methylcellulose, polycaprolactone and other materials. EXT is one of the potential approaches for printing living tissue from a hydrogel containing living cells. Also, extrusion using viscous foodstuff paste (icing, chocolate, jelly, etc.) is a preferred method for 3D printing for the food industry (Fig. 6).



Figure 6. Extrusion printing for the food industry. Source: Grup de recerca de la Universitat de Columbia - <https://www.creativemachineslab.com/digital-food.html>, CC BY-SA 4.0, <https://commons.wikimedia.org/w/index.php?curid=84202502>

Since the material for EXT 3D printing is neither photosensitive nor thermoplastic, layers do not typically solidify instantly. Thus, newly printed 3D object may need time to set and/or dry after completion as the solvent/s evaporate. It may also be prone to warping, shrinkage and deformation. Minimal layer thickness for EXT is 800 μm . This low resolution is inherent to semi-solid extrusion techniques.

- PolyJet

PolyJet is another liquid-based 3D printing technology. Like SLA, it is based on layer-by layer UV-curing of a photosensitive polymer. The ink, however, is released from the printer head in droplets



2019-1-BG01-KA203-062371

that accumulate on a building platform as directed by a designated software. PolyJet technology allows for mixing of inks, resulting in greater variety of properties for the manufactured objects.

PolyJet inks encompass great diversity of materials. Among the commonly used are ABS (different modifications), the related compound acrylic styrene acrylonitrile (ASA, more heat-resistant and UV-resistant than ABS), polycarbonate and polyetherketone ketone (PEKK). Various photosensitive materials that are used in other liquid-based 3D printing technologies may be used in PolyJet as well. There are also the specifically designed series of Vero Polyjet™ inks patented by one of the market leaders in 3D printers and materials, Stratasys. Prominent members of biocompatible PolyJet materials are MED610 and MED620, a methacrylate-based resin used in the medicine and dentistry for rapid prototyping.

Advantages of using PolyJet are the relatively high resolution (minimal layer thickness is 160 µm, allowing for creation of stable structures with complex geometry and exquisite detail) and the great variety of properties (strength, flexibility, colours, etc.). The disadvantages are (beside the common drawbacks of 3D printing described above) the inherently high levels of emission of small particles, the use of UV light and the emission of toxic fumes when curing objects manufactured with some types of inks (e.g., ASA). Printed objects may need post-processing and may be photosensitive.

- Powder based (PB) 3D printing

PB 3D printing technology is based on deposition of successive layers of powdered material and their binding by means of a liquid binder solution or fusing using other methods (see below). The powder is deposited in a powder bed or produced by a powder jet and is then sprayed or jetted with binder, irradiated with light or heated in order to achieve integrity of the object.

Minimum layer thickness may vary greatly in PB 3D printing and layers may be as thin as 20-30 µm with some materials. This very high resolution allows for creating very complex structures with exquisite detail. Generally, however, the layer thickness is about as 200 µm.

Powdered materials are usually produced by cryogenic grinding in a ball mill at a temperature that is below the glass transition temperature of the material. The grinding may be using low-temperature gases (liquid CO₂ or liquid nitrogen - dry powdering) or a mixture of low-temperature gas and organic solvents (wet grinding). In its simplest form, PB 3D printing is achievable using materials such as powdered starch or gypsum bound together with glue (Fig.7). Dyes and plasticiser compounds may be added to the binding solution in order to achieve the desired properties.



Figure 7. Rapid prototypes manufactured by powder-based 3D printing. Source: S Zillayali, CC BY-SA 3.0 <<https://creativecommons.org/licenses/by-sa/3.0/>>, via Wikimedia Commons

Disadvantages of PB-based 3D printing are related, on the one hand, on the physical properties of the objects created with this type of technology, and, on the other hand, to the need for post-processing in order to achieve maximum quality. Objects built by PB 3D are inherently highly porous and, therefore, exhibit poor mechanical resistance and may be fragile.

PB-printed objects invariably need time for evaporation of solvents. Post-processing is usually necessary in order to remove excess powder (hence a certain amount of material is always wasted) and to improve the properties of the manufactured objects (strength, colour, polish, etc.). Emission of potentially harmful small particles is a serious concern in all PB-based technologies for 3D printing.

PB has been successfully used in research, dentistry and medicine for manufacture of implants and fabrication of drugs with specific pharmacokinetic properties such as rapidly disintegrating tablets, drugs with delayed, pulsatile or extended release. Biosafety of these dosage forms is ensured by using methacrylate or ethyl cellulose as carriers of the bioactive substance.

- ColorJet

ColorJet is not exactly a technology, but a brand of powder-based 3D printers and supplies manufactured in India. It offers one of the simplest solutions in PB 3D printing. The material used is most commonly white plaster powder although plastic may also be used (e.g., the company's own patented Visijet C4 Spectrum powder). The material is layered onto a movable platform and bound by adhesive. The binder may contain different dyes, conferring different colours to the model being built.

ColorJet is most commonly used for rapid prototyping. Minimum layer thickness is about 100 μm but may vary with different materials.

2019-1-BG01-KA203-062371

Colorjet provides a budget solution where durability, brittleness and porosity are not of great concern. Therefore, it is an acceptable solution for manufacturing of models for education, research and commercial purposes (e.g., sales models) and in art. A similar process using edible powder and binder solution is commonly used in the food industry to create customised sweets and dishes.

Objects printed by ColorJet may need extensive post-processing. Plaster structures need to harden after their completion and the solvents must evaporate. Then the object is polished and impregnated with another type of binder (usually acrylic) to achieve maximum strength and to decrease surface porosity. Fumes of solvent may be an environmental concern, as well as small particles emission.

- Selective Laser Sintering (SLS)

SLS shares the common characteristics listed above for PB 3D printing. In SLS the powdered material is layered and a directed light beam generated by the printer head partially liquefies and fuses the layers to one another as the structure is being built in a process commonly termed as necking (Fig. 8).

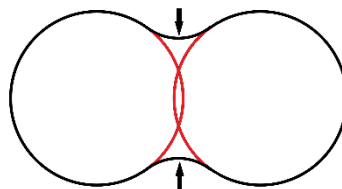


Figure 8. Formation of a 'neck' between two fusing layers (arrows). Source: Mendoza.755 - Own work, CC BY-SA 4.0, <https://commons.wikimedia.org/w/index.php?curid=76830144>

Minimum layer thickness in SLS is about 100 μm .

SLS works very well with materials with high melting point and is therefore most commonly used with metal-based inks. Plastic (polyamide, polycarbonate, polystyrene and others), thermoplastic resin, glass and ceramic powders may also be used. SLS is often used for rapid prototyping in research (especially tissue scaffolding) and in dentistry. As of now, the opportunities for development of SLS-based technology in the pharmaceutical industry are quite limited as the radiant energy and the heat generated in the course of layer fusion may seriously compromise the properties of the bioactive substance.

Powdered foodstuffs (cocoa, sugar, etc.) may be used for 3D printing in the food industry.

- Selective laser melting (SLM)

SLM is often viewed as a derivation of SLS, as it is used mainly for printing with metal and metallic alloy powders. Nevertheless, while SLS printer head laser generates just enough energy to fuse the layers



2019-1-BG01-KA203-062371

to one another, SLM uses a high-power laser that may melt completely a block of metal and then let it set in the desired shape similarly to casting. SLM produces objects that are significantly less porous than SLS and have better mechanical resilience due to homogenous internal structure. A disadvantage of SLM is that objects require polishing after their completion as it generates structures with increased surface graininess.

Electronic beam melting (EBM) is a subtype of SLM that uses similarly an electronic beam to melt metallic powders.

- Selective heat sintering (SHS)

SHS is based essentially on the same principle as SLS but the ink is thermoplastic and, respectively, the layer-by-layer fusion is obtained using heat generated by a thermal printer head. The most common material used in SHS is plastic (specifically, polyamide, but other polymers may also be used).

All objects produced by SLS or SHS are prone to warping and shrinkage. Mechanical properties of the printed objects may be inconsistent from object to object as well as from batch to batch. SLS and SHS-made objects often require post-processing, such as removal of supports and coating with sealer agents in order to decrease surface porosity.

- Multi jet fusion (MJF)

MJF 3D printing is a hybrid of the ColorJet approach (use of chemical binders) and SHS (use of heat) that works mainly with polyamide (Nylon). Basically, finely grained polyamide powder is layered onto a powder bed, sprayed or jetted with binder and is then heated selectively as instructed by a pre-programmed software. Minimum layer thickness is about 80 µm. When the object is complete, post-processing takes place (removal of excess powder, polishing, etc.). MJF produces objects that are less porous and therefore more consistent in their mechanical properties than SLS or SHS and has better capacity for reproduction of fine detail. Surface finish is also better with MJF. Chemical fumes and generation of small particles is a concern, as with all PB 3D technologies.

POST-PROCESSING REQUIREMENTS FOR DIFFERENT 3D PRINTING TECHNOLOGIES

Not all objects made by 3D printing require post-processing. Nevertheless, many do need more work after the structure is complete. Whether an object needs post-processing depends on the material, the technology, the desired aesthetical or mechanical properties, and other factors.

Generally, there are several types of post-processing of 3D-printed objects:

- Setting and/or hardening

Almost all 3D printed structures need some time after their completion in order to mature and achieve optimal mechanical properties. Objects made by extrusion-based processes (especially those that use semisolid mixtures of polymer and solvent) and powder-based processes using materials that have been impregnated with binder solutions are especially sensitive to mechanical stress immediately upon



2019-1-BG01-KA203-062371

completion. In this case post-processing includes the time for solvent evaporation and hardening. In some cases (but not always), this may be facilitated by increase of the ventilation in the premises where completed objects are kept.

- Supporting of unfinished structures

Complex structures containing overhangs may not be stable during manufacture without supports, albeit temporary. These supports need to be removed after the structure is complete, increasing the time to completion and the production costs (especially if this involves manual labour). In SLA-based 3D printing, supports for a structure printed with thermoplastic ink are most commonly made of polyvinyl alcohol. Since it is soluble, such supports may be easily removed during post-processing with a jet of water. The latter, however, requires plumbing, access to water and decontamination system for wastewater (which may increase the costs as well). Temporary support structures in SLS and SHS are usually made of unsintered material that may be vacuumed out or air-blasted away.

- Removal of excess material

This is crucial for object made by most powder-based methods. Excess powder needs to be removed using vacuum, air blast or a jet of water (again, this raises concerns about water supply and decontamination of wastewater).

- Polishing

Polishing is a must for improving of the mechanical characteristics of mechanical parts (especially those subjected to movement and friction) and objects with increased graininess of the surface (such as those produced by selective laser melting). Objects with aesthetical properties may also need polishing.

- Sealing of surface pores

This is an important post-processing step for technologies that yield objects with increased porosity, such as SLS or SHS. Surface pores may be sealed with impervious coatings such as cyanoacrylate coatings or by hot isostatic pressing.

- Painting

This is not commonly needed, as most 3D printing processes use differently coloured materials or differently coloured binders.

WHICH PROCESS FOR WHICH? BASIC CONSIDERATIONS WHEN SELECTING 3D PRINTING TECHNOLOGY SUITED FOR A PARTICULAR APPLICATION

While all 3D printing technologies may have overlapping applications (e.g., rapid prototyping), there are factors that may weigh in when a 3D printing technology is selected for a particular project or a specific industrial solution. Main considerations are:

- Type of material that may be used to print an object. Many 3D printers on the market may use a variety of different materials. Architectural, sale and exhibition models as well as models made for



2019-1-BG01-KA203-062371

educational purposes may conveniently be created by ColorJet, SLA and FDM technologies that use plaster or different types of plastic. Small desktop machines that serve hobbyists and artists are usually based on FDM technology. SLS/SLM and SHS are usually employed when the objects must be made from metal and metallic alloys.

- Availability of 3D printing machine. Usually, orders for a particular machine are queued and work in a 'first come, first served' fashion. In regions where 3D printers are hardly available, this factor may be of prime importance, prevailing over the type of material.
- Availability of material. Plastic is versatile, available and, except for high-performance polymers, relatively cheap, while metal may be more costly.
- Costs for production per object. These are related to the technology used for creation of objects, the material used and the needs for post-processing. It was already mentioned that, unlike casting, scaling up in 3D printing does not always bring the unit price down. The price per object includes not only the costs for manufacturing but also the post-processing costs (if it is needed) as well as the costs incurred by measures for decreasing environmental pollution. Among commonly used PB technologies, SHS is a more budget-oriented solution than SLS as purchase and maintenance of a thermal printer head is generally less expensive than a laser printer head. Objects manufactured by SHS are therefore generally cheaper than those made by SLS and the process may easily be scaled down for small-sized orders.
- Rapidity of manufacture, including post-processing and the time for queueing of orders. Small objects may be printed in a matter of minutes to hours. The time for printing increases with the size of the objects to a whole day and, ultimately, with very large objects, up to a several days, although the latter is rare. Post-processing times may also be a concern. Extrusion technologies does not typically need finishing, but the object may need time to set and harden. ColorJet-made objects need time for drying and setting. Powder-based technologies almost invariably need time for post-processing and it increases the costs as well as the time for manufacturing.
- Properties of the end product (mechanical properties, aesthetical value)

Plastic 3D printing ink may come in many different varieties with different mechanical properties. Depending on the purpose of the use of the object, the preferences may be for plastic (lightweight and, at the same time, durable, easily dyed, easily polished, thermo- and photocurable) or for metal (resilient, resistant to UV and heat, colouring and polishing may need special equipment and processes).

Among the plethora of varieties of plastic, different polymers with different mechanical resilience are available. Generally, SLA printing allows for finely polished structures with exquisite detail that are, nevertheless, quite brittle. It is therefore unsuited for applications where durability is crucially important. FDM yields significantly more durable objects than SLA, yet there are different grades of mechanical resilience depending on the material used. Generally, PVA, ABS and PLA are "commodity" inks for FDM. These are relatively cheap but the resulting objects are prone to warping. Objects printed with PLA are also quite brittle. ABS-printed objects are photosensitive. Use of polyurethane yields parts with significant degree of flexibility. Next step in FDM quality is PC, PETG and PPE. These materials are used mainly as engineering materials for manufacture of mechanical parts and tools. PETG is also food-safe. High-resistance FDM inks are PEI and PEEK which are used for parts



and devices that need to exhibit very high resistance, such as moving and/or strain-bearing parts and when thermal stability is crucial. All 3D printed objects might be prone to delamination under stress, but PEEK printed objects exhibit excellent layer-to layer adhesion and are therefore very durable. It is, however, quite expensive, and as the melting point is higher than of the other FDM inks, the printer needs to have the capability for heating the nozzle to high temperatures.

3D printed drug dosage forms and tissue scaffolding have specific requirements that are generally met by extrusion methods (especially semisolid extrusion such as EXT) and some of the powder-based technologies (but not these that require large amounts of radiant or infrared energy such as SLS and SHS). As minimal layer thickness for EXT is almost 1 mm, the printed structures are coarser and less detailed.

Powder-based methods have very high degree of resolution and therefore confer better reproduction of fine detail. This is especially true for SLS (it, however, works mainly with metallic powders) but MJF (works readily with plastic) also compares favourably in terms of resolution.

The structures produced by most PB technologies are also durable (except for ColorJet-printed plaster objects) but porosity may compromise their mechanical properties. When the latter is a serious issue, SLM may be a solution, provided that proper post-processing is carried out.

- Environmental concerns (related to manufacture or post-processing or the biodegradability of discarded objects).

Extrusion-based and some of the PB technologies generate solvent fumes. Powder-based technologies generate large amounts of small particles. All these have significant potential harmful effects and need to be filtered out and/or decontaminated.

COMMONLY USED SOFTWARE FOR 3D PRINTING

3D printing uses previously collected data that has been stored on a digital information carrier. In the course of 3D printing of a complex object, the momentary changes in the pattern of deposition of the ongoing layer are dictated by the information of specific cross-sections in the digital file representing how exactly the structure must look like at this particular point. Thus, structures with simple patterns may be represented by fewer cross-sections while digital files for structures with intricate patterns ought to contain more cross-sections, indicating where and how the structure is altered. Every cross-section represents a closed polygon and corresponds to a different position along the z axis. Cross-sections must not overlap or intersect and, at best, must be without voids in between. Small discrepancies may be cleared out by the software without significant shape and structure alterations.

Computer-assisted design (CAD) images are most commonly used for 3D printing. Those may be the conventional 2D DWG images generated by AutoCAD although these need to be converted to 3D by designated software, e.g., Sketchup Pro. Stereolithography file format (Standard Tessellation Language, STL) was the first to be specifically designated for additive manufacturing. It was developed and adapted for use in the 1980-ties by 3D Systems, a company founded by Charles Hull, the inventor of SLA. Apart from AutoCAD, commonly used CAD software capable of exporting or converting data



2019-1-BG01-KA203-062371

to STL format are Alibre, Mechanical Desktop, I-DEAS, SolidDesigner, ProE and others. Online converters are also readily available, such as AnyConv (<https://anyconv.com/stl-converter/>).

Images generated by a 3D scanner or by photogrammetry of a typical 2D digital image (taken by a digital camera) may also do for additive manufacturing. Depending on the source of the data for the object, the final result may be more or less true to the initial image. CAD images usually result in objects with significantly less errors than other types of digital data. These errors may be corrected later. There are many different brands of software for photogrammetry, mainly Microsoft Windows-based (Zephyr3D, PhotoModeler, PhotoSynth and others) but there are some may use other operating systems such as Linux and MacOS (e.g., Pix4D Mapper, Metashape, OpenDroneMap and others). A detailed list of commonly used photogrammetry software with their basic characteristics may be viewed here:

https://en.wikipedia.org/wiki/Comparison_of_photogrammetry_software

Online tools for photogrammetry are also available, such as the mentioned above AnyConv (<https://anyconv.com/stl-converter/>).

3D printing design guidelines

In order to print an object, if a STL file is not available, it must be output from a piece of software such as AutoCAD or others (see above). Most professional 3D-modeling software is proprietary, but free software is also available, mainly for education purposes for beginners in the field and for hobbyists (FreeCAD, 3D Slash, TinkerCAD, Vectary, SketchUp, Leopoldy, Sculptris, Rhinoceros and others). Then the resulting STL file is used by another piece of ‘slicer’ software, such as Repetier, OctoPrint, Z-Suite, Tinkerine Suite, Cura, PrusaSlicer, Simplify3D and others to generate a file containing series of thin layers. The resulting file is used by the 3D printer host to generate a 3D object. Some pieces of slicer software function also as printer hosts, such as Cura, 3DPrinterOS, OctoPrint, MatterControl and others. While most professional 3D modelling software is usually stand-alone, free software may be available as a plugin and is much more likely to be available for operating systems other than Windows.

STL files contain raw mesh data based on triangulations of the surface of CAD models. Thus, the surface of every facet of the object is represented by a polygon mesh that must conform to a set of rules. The basic rules are four, namely:

- The ‘vertex to vertex’ rule - each triangle must share 2 vertices with its neighbouring triangles;
- The ‘right-hand rule’ - the vertices must be listed in counter-clockwise fashion;
- The ‘pointing outwards’ rule - the direction of normal vector should point outwards;
- The ‘all-positive rule’ - all the coordinates of the triangle vertices must be represented by positive numbers.

An example may be viewed in Fig. 9.

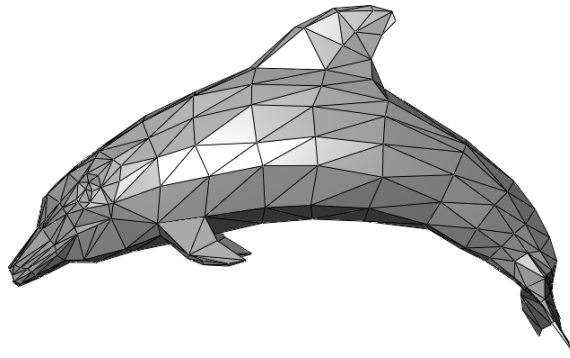


Figure 9. A polygon mesh representing a 3D image of a dolphin. Source: Chrschn, Public domain, via Wikimedia Commons

STL files may store information in binary encoding or in ASCII encoding. Files in binary are, generally, smaller and may be easily shared, while ASCII files are used to manually read and check the STL file, e.g., when testing new CAD interfaces.

Complex objects may be a challenge to STL files as there are too many surfaces to be taken in to account in the calculations. In addition, the early STL files had very limited properties for representing colour, texture, material and internal structure (although this was resolved later). Some types of software, such as the prototypic AutoCAD may only export designs of solid objects to STL. Non-adjointing surfaces (e.g., containing gaps or overlaps) are difficult to process or adapt in STL files and would not print. Thus, in 2006 an improved version of STL formats was released that eventually was transformed in another, more versatile CAD file format, the Additive Manufacturing File format (AMF). Nevertheless, many 3D printers used today, especially those that work in the small scale, will use STL files.

AMF files can readily represent internal structure using use face-vertex polygon layout and curved triangles to define a discrete unit of volume of material. Multiple volumes may be specified for a single object. AMF files also contains information about the material, the colour and texture of the printed object or its parts and the order for printing.

Jonathan Hiller, one of the inventors of the AMF file format hosts a detailed and user-friendly site providing CAD software or 3D modelling and tutorials: <https://sites.google.com/site/jonhiller/software>.

Free architectural CAD drawings for AutoCAD and 3D modelling software are readily available on the Internet, e.g. <https://www.arcad.com/>, <https://www.tinkercad.com/>, <https://www.sketchup.com/> and others. A free 3D CAD image is presented in Fig. 10.



2019-1-BG01-KA203-062371

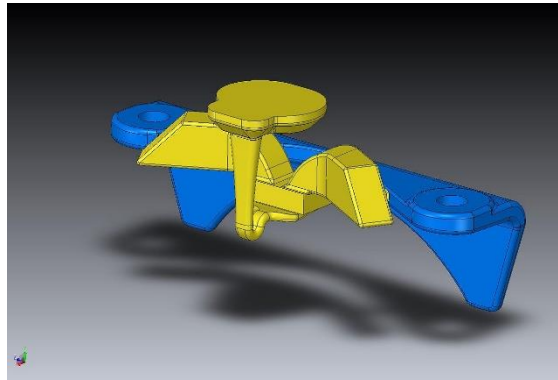


Figure 10. A 3D CAD model for use in 3D printing. Source: OKFoundryCompany from Richmond, USA, CC BY 2.0 <<https://creativecommons.org/licenses/by/2.0>>, via Wikimedia Commons

Free STL files, 3D printer files and 3D printer designs are available for use from www.thingiverse.com, www.youmagine.com, www.myminifactory.com, <https://library.zortrax.com/>, <https://www.cgtrader.com/>, <https://cults3d.com/en>, <https://www.youmagine.com/>, <https://www.turbosquid.com/Search/3D-Models/free/stl>, and other sites.

STL editor software is available for multiple sources such as <https://www.meshlab.net/>, <https://www.blender.org/>, <https://www.freecadweb.org/>, <https://www.3dslash.net/index.php>, <https://www.sketchup.com/>, and others.



References

- Kodama H. 1981. Automatic method for fabricating a three-dimensional plastic model with photo-hardening polymer. *Rev Sci Instrum*, 52(11): 1770–73.
- Melchels FP, Feijen J, Grijpma DW. 2009. A poly(D,L-lactide) resin for the preparation of tissue engineering scaffolds by stereolithography. *Biomaterials*, 30(23-24):3801-9.
- Shelby A. Skoog, Peter L. Goering, Roger J. Narayan. 2014. Stereolithography in tissue engineering. *J Mater Sci Mater Med*, 25: 845–56.
- Alhnan A, Okwuosa M, Chijioke T, et al. 2016. Emergence of 3D Printed Dosage Forms: Opportunities and Challenges. *Pharm Res*, 33 (8): 1817-32.
- Goyanes A, Buanz AB, Basit AW, et al. 2014. Fused-filament 3D printing (3DP) for fabrication of tablets. *Int J Pharm*. 2014;476(1–2):88–92.
- Wang J, Shaw LL, Cameron TB. 2006. Solid Freeform Fabrication of Permanent Dental Restorations via Slurry Micro-Extrusion. *J Am Ceram Soc*, 89 (1): 346–9.
- Khaled SA, Burley JC, Alexander MR, et al. 2014. Desktop 3D printing of controlled release pharmaceutical bilayer tablets. *Int J Pharm*, 461(1–2):105–11.
- Azad MA, Olawuni D, Kimbell G, et al. 2020. Polymers for Extrusion-Based 3D Printing of Pharmaceuticals: A Holistic Materials-Process Perspective. *Pharmaceutics*, 12(2):124.
- Chua CK, Leong KF, An J. 2014. 1 - Introduction to rapid prototyping of biomaterials. Woodhead Publishing.
- Sun Y, Soh S. 2015. Printing Tablets with Fully Customizable Release Profiles for Personalized Medicine. *Adv Mater*, 27(47):7847-53.
- Edelmann A, Dubis M, Hellmann R. 2020. Selective Laser Melting of Patient Individualized Osteosynthesis Plates-Digital to Physical Process Chain. *Materials (Basel)*, 13(24):5786.
- Tan W, Gao C, Feng P, et al. 2021. Dual-functional scaffolds of poly(L-lactic acid)/nanohydroxyapatite encapsulated with metformin: Simultaneous enhancement of bone repair and bone tumor inhibition. *Mater Sci Eng C Mater Biol Appl*, 120:111592.
- Schubert C, van Langeveld MC, Donoso LA. 2014. Innovations in 3D printing: a 3D overview from optics to organs. *Br J Ophthalmol*, 98:159-161.



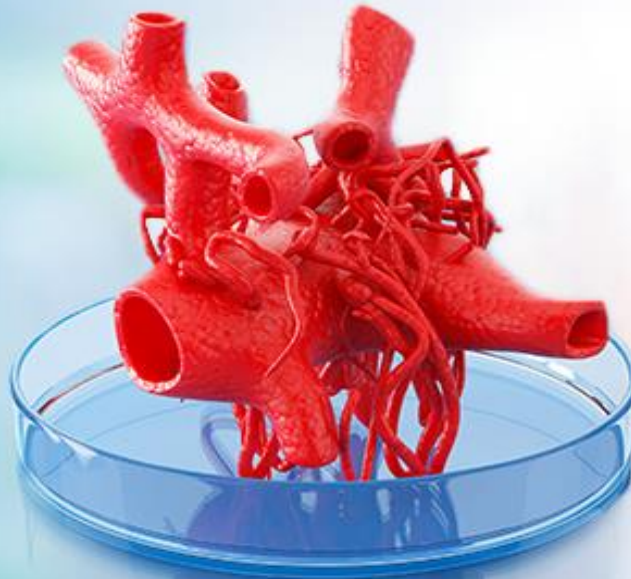
2019-1-BG01-KA203-062371

Hiller J, Lipson H. 2009. STL 2.0: A Proposal for a Universal Multi-Material Additive Manufacturing File Format". Solid Freeform Fabrication Symposium (SFF'09), Aug 3-5 2009, Austin, TX, USA.



Project website: www.digit-biotech.eu

The European Commission's support for the production of this publication does not constitute an endorsement of the contents, which reflect the views only of the authors, and the Commission cannot be held responsible for any use which may be made of the information contained therein.



LO2 THE APPLICATION OF 3D PRINTING AND ICT FOR GREEN PRODUCTS AND PROCESSES

Advanced level

AUTHOR:
VENTSISLAVA PETROVA



Contents

Application areas of 3D bioprinting	3
Tissue engineering and regenerative medicine	4
1. Bone tissue	4
2. Cartilage tissue	5
3. Cardiac tissue	6
4. Liver tissue	7
5. Lung tissue	7
6. Neural tissue	8
7. Pancreas tissue	9
8. Skin tissue	10
9. Vascular tissue	11
10. Other tissue types	12
Transplantation and clinics	12
Pharmaceutics and high-throughput screening	13
Cancer research	15
Concluding remarks and future outlook	16
References	18



Application areas of 3D bioprinting

3D bioprinting is an evolving technology with applications throughout the complete range of biomedical research. The method employing a biological substrate or ‘bioink’, made of structural components, growth media, and cells, can produce on 3D printing equipment biological structures that closely imitate the structure of cells in living organisms. Using the same manufacturing model as traditional 3D printing, a number of 2D slices are divided from a 3D model by a computer to print a correct biological composition layer-by-layer with a precise arrangement of tissues and cells. 3D bioprinting approach gives an opportunity to solve issues in drug development, regenerative medicine, organ donation, and cancer treatment. This emerging technology offers great accuracy on spatial placement of cells, proteins, DNA, drug particles, growth factors and biologically active substances to guide better tissue formation. This powerful technology seems to be more promising for improving tissue fabrication toward physiologically relevant tissue constructs, tissue models, tissues and organs and organs-on-a-chip models for medicine and pharmaceuticals.

Bioprinting technology has a wide application in various areas such as tissue engineering and regenerative medicine, transplantation and clinics, drug screening and high-throughput assays and cancer research (Fig. 1). Bioprinting for tissue engineering and regenerative medicine has been studied for more than a decade, and anatomically correct cell-laden constructs and scaffolds have been produced for various tissue types: from connective and epithelial tissues to muscle and nervous tissues. With its great advantage in modelling and precisely placing multiple cell types, bioprinting has overcome one of the major shortcomings of traditional scaffold fabrication techniques and has facilitated construction of native-like tissues with a heterocellular microenvironment. Although most of the effort has been targeted toward the fundamental science behind major bioprinting techniques such as laser-, droplet- and extrusion-based bioprinting, a considerable attention has recently been given to bioprinting for functional tissue production. Particularly, considerable work has been devoted to bioprinting for transplantation. As additional progress has been made in biomaterials, cell and transplantation technologies, bioprinting will translate from bench to bedside when authorized for human use and has a multitude of advantages in operating rooms in the near future. Before transitioning into clinical practice, bioprinting has already made a great jump in pharmaceutical use because it does not require any regulatory approvals and there is currently an emerging bioprinting market for tissue fabrication for drug testing and high-throughput assays. Bioprinted tissue models (e.g. liver), fabricated from multiple cell types and facilitating a complex heterocellular physiological environment, have been used in drug screening. Moreover, bioprinting has recently been applied in cancer research to investigate cancer pathology, growth and metastasis in a physiologically relevant microenvironment. Here, a comprehensive review is presented of the application areas of bioprinting technology and in-depth discussion is presented on successfully bioprinted tissue types for tissue engineering and regenerative medicine, transplantation and clinics, drug screening and high-throughput assays, and cancer research. For each application area, advantages and limitations of existing technologies are discussed.

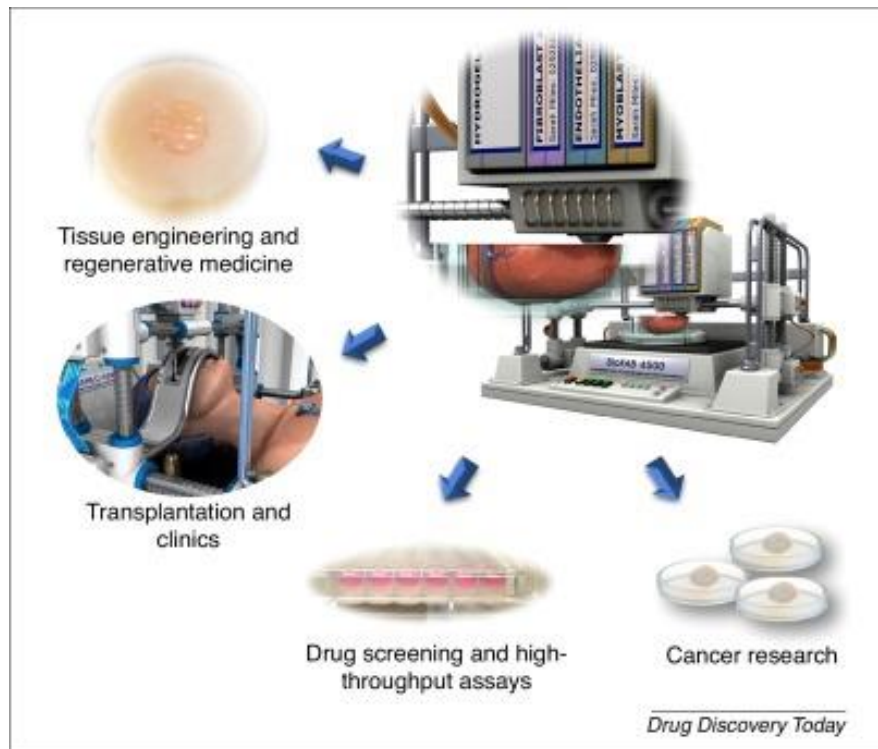


Figure 1. Application areas of bioprinting technology

TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Bioprinting of functional organs at clinically level remains elusive because there are several challenges such integration of the vascular network from arteries and veins down to capillaries, combination of various cell types to recapitulate complex organ biology and limited structural and mechanical integrity and long-term functionality. Despite these challenges, a large variety of tissues have been successfully bioprinted such as thin or hollow tissues, for example blood vessel, or tissues that do not need vascularization such as cartilage.

1. Bone tissue

Bone is an essential tissue of the body and a key component of the musculoskeletal system. Generally, it overcomes insult and degeneration through its highly regenerative capabilities. However, in some cases bone fails to properly heal and treatment is required. The existing standard of care therapies



2019-1-BG01-KA203-062371

has significant weaknesses. Innovations in bioprinting and tissue engineering hold much promise for improving both the quality and efficacy of bone reconstructive therapies. Bioprinting, theoretically, does not yet occur because custom resorbable scaffolds are not currently constructed with growth factors or autologous cells. However, latest investigations have been rapidly improving the 3D printing techniques as well as 3D printable materials with properties finely tuned to reconstruct patient-specific defects. This research seems to be leading toward a future of resorbable scaffolds that incorporate appropriate growth factors and osteoprogenitor cells. Time will reveal whether these therapies will become widely available and standard-of-care.

In a recent study, some authors used a thermal inkjet bioprinter to build poly(ethylene glycol) dimethacrylate (PEGDMA) scaffolds. Nanoparticles of bioactive glass and hydroxyapatite (HA) were used for co-printing of bone-marrow-derived human mesenchymal stem cells (hMSCs) under simultaneous polymerization. This approach enabled uniform distribution of hMSCs compared with manually pipetted hMSCs, which accumulated at the bottom of the scaffold because of gravity. The bioprinted structures encapsulating hMSCs and HA exhibited the highest cell viability, collagen production and alkaline phosphate activity with increased compressive modulus after 21-day culture *in vitro*. Bioprinting HA particles were also carried out for *in situ* bioprinting purposes, where a laser-based bioprinting system was employed to deposit HA nanoparticles into mouse calvaria defects.

In another study, heterocellular tissue constructs made of Matrigel™ and alginate hydrogels were bioprinted. Endothelial progenitor and multipotent stromal cells were bioprinted in a spatially controlled manner. The obtained constructs were subcutaneously implanted into immune-deficient mice. By integrating osteoinductive biphasic calcium phosphate microparticles, multipotent stromal cells were differentiated into an osteogenic lineage and accelerated bone formation in 6 weeks. In addition to osteoinductive materials, addition of growth factors is also crucial in stem cell differentiation in bone tissue engineering. It was demonstrated that addition of bone morphogenetic protein (BMP)-2 has important role for the stem cell fate. Using inkjet bioprinting of patterned BMP-2 on fibrin-coated coverslips primary-muscle-derived stem cells were bioprinted. It was shown however, that they were differentiated toward osteogenic lineage on the pattern even if they were treated with myogenic differentiation conditions.

2. Cartilage tissue

Over the last two decades significant progress has been made not only in the field of bone tissue engineering but also in the cartilage one. Consequently, there is a true potential that techniques to regenerate rather than replace damaged or diseased bones and joints will one day reach the hospital. However, several key challenges must still be addressed before this becomes a reality. These mainly include vascularization when large bone defect repair is required. Other important issues are the necessity of usage of engineering complex gradients for bone-soft tissue interface regeneration and recapitulating the stratified zonal architecture present in many adult tissues such as articular cartilage. However, existing cartilage engineering approaches have great potential for precise construction of cells



2019-1-BG01-KA203-062371

and biomaterials with sophisticated patterns. They can mimic native tissues with zonally differentiated cells and extracellular matrix (ECM) structure.

Laser-based bioprinting of stem-cell-differentiated chondrocytes was also attempted. This strategy is based on a computer-aided biofabrication technique with the assistance of LIFT. Porcine-bone-marrow-derived mesenchymal stem cells (MSCs) with high viability were successfully bioprinted. They maintained their functionality and differentiation ability into osteogenic and chondrogenic lineages. Inkjet-based bioprinting has also been applied in cartilage tissue engineering as well as for cartilage defect regeneration. HP desktop printer was modified, and human chondrocytes loaded in PEGDMA hydrogel were bioprinted. Thus, fabricated cartilage construct had mechanical properties and biochemical composition close to native cartilage. Most recently a hybrid bioprinting method to fabricate mechanically improved cartilage tissue constructs by combining 3D bioprinting and electrospinning techniques was created. In that study, electrospinning of polycaprolactone (PCL) fibres together with inkjet bioprinting of rabbit elastic chondrocytes in fibrin–collagen hydrogel was shown.

Despite the great success in cartilage tissue bioprinting, fabricating of zonally stratified articular cartilage tissues with different structural, biomechanical and biological properties is still a challenge and further progress is required to produce articular cartilage tissue constructs with zonal differentiation including more horizontal and thinner collagen fibres with high cell density in the superficial zone, and relatively vertical and thicker collagen fibres with less cell density in the deeper zones.

3. Cardiac tissue

Cardiovascular diseases are the primary cause of death in the world. In this respect the cardiac and heart valve tissue engineering is from utmost importance. In the past decade a significant progress has been made in studying the native properties of the heart for cardiac regeneration and repair using a variety of strategies, including concepts of biomaterials, micro- and nanotechnologies, and decellularization. However, main difficulty in cardiac tissue engineering is incorporating the complex, heterogeneous micro- and macro-cardiac tissue structures for different cell types to adhere, proliferate, and ultimately repair and remodel damaged parts. 3D bioprinting has appeared as a strategy to address this challenge by providing the technology to construct high-resolution scaffolds with heterogeneous biochemical and mechanical characteristics. Though, limited attempts have been made in bioprinting of cardiac tissue models. It has been demonstrated extrusion-based bioprinting of tissue spheroids comprising human vascular endothelial cells (HUVECs) and cardiac cells obtained from myocardial tubes of chicken embryos. Other approach has been based on bioprinting a cardiac tissue construct in a half-heart shape with connected ventricles using inkjet-based bioprinting. Laser-induced forward-transfer (LIFT) has also been utilized to pattern HUVECs and hMSCs in a geometrically defined pattern on polyester urethane urea and the produced samples were transplanted to the infarcted zone of rat hearts after LAD ligation. Eight weeks post-transplantation, it has been observed increased vessel formation compared with randomly bioprinted cells as control groups and the resulted myocardium patch provided considerable functional improvement.



2019-1-BG01-KA203-062371

Other important direction is the engineering of heart valves because they do not possess regeneration capacity and dysfunctional heart valves, if the damage or disease is detrimental, need to be replaced by mechanical or biological prosthetic equivalents. However, fabrication of such replacement valves is restricted by thrombogenicity and calcification. Despite its important role in the cardiovascular system, only a limited amount of work has been performed in the bioprinting of heart valves. The first-time bioprinting of a heart valve has been achieved via using a dual-head bioprinter modified from a Fab@Home printer. In another study, dual-nozzle bioprinting of composite alginate-gelatin hydrogel was used for the construction of thin hydrogel discs and spatially bioprinting of aortic root sinus smooth muscle cells (SMCs) and aortic valve interstitial cells (VICs). In a recent study, the same group performed bioprinting of composite hydrogels using methacrylated hyaluronic acid (Me-HA) and methacrylated gelatin (Me-Gel) loaded with human aortic valvular interstitial cells (HAVICs). Obtained samples were designed in tri-leaflet valve-shape and the cells bioprinted with increased Me-Gel concentration exhibited better spreading.

4. Liver tissue

The liver is the largest gland in the human body. It plays a key role in metabolism, bile production, detoxification, and water and electrolyte regulation. The toxins or drugs that the gastrointestinal system absorbs reach the liver first before entering the bloodstream. Liver disease is other leading causes of death worldwide. Hence, an *in vitro* liver tissue model that simulates the main functions of the liver can be an important platform for investigating liver diseases and developing new drugs. Currently, the newly developing 3D bioprinting technologies have the ability to assemble *in vitro* liver tissue models both in static scaffolds and dynamic liver-on-chip manners.

The first-time bioprinting of human-induced pluripotent stem cells (hiPSCs) was performed through stimulating hiPSCs and differentiating them into hepatocytes for liver micro-organ engineering. Additionally, to bioprinting liver tissue structures, liver carcinoma HepG2 immortal cells were bioprinted in larger tissue models.

In fact, 3D-bioprinting liver tissue scaffolds and constructing liver-on-chips can significantly help for the development of *in vitro* liver tissue models. By employing 3D bioprinting technology in liver-on-a-chip fabrication, the construction of *in vitro* liver tissue models will become more efficient and cost-effective. Thus, a powerful platform will be provided for screening drugs and determining the molecular mechanism underlying liver diseases.

5. Lung tissue

3D bioprinting increases the possibility of reproducibly creating complex macro- and microscale biostructures using multiple different cell types. This is promising strategy for fabrication of multilayered



2019-1-BG01-KA203-062371

hollow organs, which is a big challenge for the more traditional tissue engineering techniques. Currently, the main progress for the pulmonary system has been limited to the trachea. The available techniques for tracheal graft construction are valuable foundations to whole lung bioprinting. Generally, most 3D bioprinting approaches for tracheal production combine biological hydrogels seeded with cells surrounding a sturdy polymer structure.

Generally, the lung is classified surgically as a solid organ. However, for the purposes of tissue engineering it is more useful to consider the entire respiratory tree as a branching set of tubes. Thus, the progress and difficulties detected in bioprinting of other tubular organs can help in bioprinting of the lung. The attempts for lung bioprinting are new and there are only few attempts exploring the synthesis of a lung tissue model. It was recently achieved bioprinting of an *in vitro* air-blood barrier model through development of a zonally stratified tissue construct in a few layers. First, a thin layer was bioprinted. It serves as a basement membrane. Then a single layer of endothelial cells was bioprinted facilitating the attachment of cells on the next layer. Later, a new layer was added on top of the previously assembled construct followed by bioprinting of a single layer epithelial cells.

Working toward a more complex geometry, some authors has developed a distal lung model with vascular and airway spaces. They used poly(ethylene glycol) diacrylate and a stereolithographic printer and manage to create a “breathing model” with tidal air ventilation and blood flow. Applying this model, they investigated the pulmonary transport by measuring blood oxygenation entering and leaving the construct. Though the printed vasculature was limited to 300 µm, it is still a large step towards creating clinically relevant lung tissues.

6. Neural tissue

The human nervous system is extremely complex physiological network that is intrinsically challenging to analyse because of difficulties to obtain primary samples. One of the powerful alternatives to study nervous system development, diseases, and regenerative processes, are the animal models. However, they are unable to address some species-specific characteristics of the human nervous system. More and more sophisticated *in vitro* models of the human nervous system have been developed, but still require further advances to better simulate microenvironmental and cellular features. The field of neural tissue engineering is rapidly implementing new tools that enable researchers to precisely monitor *in vitro* culture conditions and to better model nervous system formation, function, and repair.

However, the work done in the framework of bioprinting for neural tissue construction is quite scarce. Some of them has been based on the bioprinting of C17.2 cells on a collagen layer next to a fibrin disk loaded with VEGF. Using this model, the effect of vascular endothelial growth factor (VEGF) on the proliferation and migration of murine neural stem cells (C17.2) has been analyzed. The study demonstrated that neural cells migrated toward VEGF-releasing fibrin gel and proliferated successfully. Recently, other investigations presented bioprinting of a thermoresponsive polyurethane hydrogel with



2019-1-BG01-KA203-062371

tunable stiffness and gelation ability at 37°C without the requirement for a crosslinker. The effectiveness of the bioink was achieved by loading it with neural stem cells and injecting it into a zebrafish embryo neural injury model. Obtained results revealed that the injected gel rescued the function of the impaired nervous system in 6 days.

A scaffold-free approach was also used, where a pellet of Schwann cells (SCs) and bone marrow stem cells (BMSCs) were extruded within a 3D-printed agarose. After cells aggregation in the agarose a nerve tissue graft with three lumina in each was formed. The constructed grafts were then implanted into mice, and their histology and functionality were assessed. The number of samples were limited for drawing a definitive conclusion about the performance of the 3D bioprinted grafts with respect to commercially available collagen ones. However, the presented case demonstrated a proof-of-concept for bioprinting nerve grafts.

7. Pancreas tissue

Studying and treating pancreatic diseases, such as diabetes and pancreatic cancer, is hindered by the restricted access to pancreatic tissue *in vivo*. However, three-dimensional (3D) tissue models, which accurately imitate the native organ, have great potential in biomedical applications, by both providing effective platforms for investigating tissue development and homeostasis and for simulating diseases in pharmaceutical testing. Unfortunately, primary pancreatic β -cells do not easily survive *in vitro* and only a very few attempts have taken place to differentiate β -cells from human stem cells. In this regard the regeneration of pancreas tissue is primarily exemplified to the extent that β -cells from mouse lines or insulinoma cells have been used to produce pancreatic islets. A limited amount of work has been done in the context of bioprinting for pancreatic tissue fabrication. Recently, human and mouse islets as well as rat insulinoma INS1E β -cells were encapsulated within alginate or alginate-gelatin hydrogels and bioprinted them in dual-layer scaffolds. In addition, recent work demonstrated the microfabrication of scaffold-free tissue strands (with strong expression of insulin) for extrusion-based bioprinting, where tissue strands were made of rat fibroblasts and mouse insulinoma TC-3 β -cells in the core and shell, respectively. An innovative approach was proposed comprising the use of tissue strands for scale-up the tissue bioprinting process.

This experimental attempts in the advanced technological field of bioprinting will pave the way towards new technological opportunities of growing functional tissues and organs in a laboratory. It was expected that the knowledge and models generated by the research in the field of bioprinting will not only stimulate major technological developments in tissue-engineering, but will also open radically new possibilities in medicine, such as studying pancreatic diseases *ex vivo* in fabricated tissue, developing new drugs, and, possibly facilitating the replacement of injured or diseased tissue.



2019-1-BG01-KA203-062371

8. Skin tissue

Skin is the outermost protecting cover of human body and is in direct contact with the external environment. That makes it highly vulnerable to injury. Skin defects or wounds may result from trauma, skin diseases, burn or removal of skin during surgery. Such conditions require urgent therapeutic interventions to repair the structure and function of the skin and allow the normal mobility of the patient. Furthermore, existence of wounds can lead to the bacterial contamination and related complications if not treated immediately. Moreover, even minor deformities can create psychological distress on the affected individuals, especially to children. Currently, the best alternative of skin tissue engineering is the use of autografts. However, limitations exist including the amount and size of available grafts, creation of a secondary wound and other risks. Employing other types of skin grafts such as allografts and possibly xenografts are associated with the risks of immune reactions and transmission of diseases besides some ethical and cultural issues. There is also a possibility to use wound dressing materials such as those based on polymers or their combinations with other substances. However, they are not living and hardly can be cellularized and replaced by native tissue. In this regard, tissue engineering holds great promises for improving the treatment of skin defects, but solutions should be found for the challenges such as development of multi-layered native skin architecture and vascular networks. Some solutions has already been proposed where besides biomaterial, living cells, biological or chemical signals are being used with the purpose of forming functional skin. However, such conventional tissue engineering methods suffer from several problems like non-homogeneous distribution of cells, failure to integrate and vascularize the construct upon its implantation, subsequent rejection of the implanted biomaterial along with formed skin. Although, some tissue engineered skin products are in market, many limiting factors are encountered with a major and critical one for clinical success being the vascularization through the skin substitute.

A variety of tissue engineering approaches have been used in skin tissue construction and different tissue substitutes have been created including autologous split-thickness skin graft (gold standard), allografts, acellular dermal substitutes and cellularized graft-like commercial products. Bioprinting of skin tissue has been presented by using an eight-channel valve-based bioprinter, where a 13-layer-tissue construct was bioprinted employing collagen hydrogel. Keratinocytes were bioprinted on top of alternating layers of human foreskin fibroblasts and acellular collagen layers. The resulted constructs exhibited densely packed cells in epidermis layers as opposed to the dermis with low density of cells and less ECM deposition. In addition to droplet-based bioprinting, laser-based bioprinting has also been applied for bioproduction of skin tissue substitutes. In a recent study the effect of bioprinting endothelial cells within skin substitutes on formation of macrovasculature during new tissue remodeling was analysed. The approach included encapsulation of neonatal human dermal fibroblasts and epidermal keratinocytes (NHEKs) in collagen. Next the dermis layer is laid down followed by patterning human dermal microvascular endothelial cells (HMVECs) on the dermis layer of the construct. The process was completed by wrapping the fibrin layer with collagen-laden NHEKs. Then, the produced skin substitutes were implanted on the dorsa of mice and compared with commercially available skin substitutes as a control. Obtained results showed that bioprinted HMVECs formed microvessels and the implanted constructs generated contraction compared with the control groups. Moreover, in other studies it was



2019-1-BG01-KA203-062371

demonstrated *in situ* bioprinting of stratified skin substitutes by alternating layers of fibrinogen–collagen and thrombin loaded with amniotic-fluid-derived stem (AFS) cells. With this approach, skin substitutes were 3D bioprinted directly onto full thickness wounds on pigs and repaired the native skin more closely than control groups. Despite the efforts in skin tissue bioprinting, bioconstruction of skin substitutes that virtually simulate native skin is still a challenge because integrating sweat glands and hair follicles has remained elusive.

9. Vascular tissue

Three-dimensional (3D) printing technology has rapidly become from a futuristic technology to a promising science which is believed to revolutionize clinical practice by making available customized equipment, organ models for surgical practices, organ replacement parts, and modern drugs. Though originally this technology was intended to provide surgical models and prototypes, scientists have rapidly advanced the technology to produce tissue constructs for regenerative medicine. However, large-scale implementation has still been constrained due to the anatomical complexity, compositional (cellular and extracellular) diversity, and vascularization of native tissues.

Vascular tissue synthesis has been performed by different bioprinting approaches including extrusion, droplet and laser-based bioprinting. In extrusion-based bioprinting, a broad spectrum of extrusion techniques has been utilized. Some scientists use coaxial-nozzle extrusion, where hydrogels including sodium alginate and chitosan were bioprinted directly in tubular form with encapsulated cells. During co-axial (core-shell) flow, ejected crosslinker (flowing through the core) make a contact with the precursor hydrogel solution (flowing through the shell), and accelerated the gelation and formation of tubular constructs. That approach facilitates direct bioprinting of vascular structures in a practical manner. There are also other direct vascular tissue bioprinting strategies, such as bioprinting droplets of cell-laden hydrogels layer-by-layer using inkjet-based bioprinting. Using bottom-up construction, inkjet-based bioprinting allowed building of branched tubes in horizontal and vertical directions. A related approach was also carried out using laser-based bioprinting. In all the above-mentioned studies, scaffold-based techniques are utilized. However, some investigations are based on a scaffold-free approach in bioprinting vascular tissues, where tissue spheroids were fabricated one by one and self-assembled into larger tissue units.

Though the sphere of 3D bioprinting has brought tremendous advances in producing vessel structures with sound structural and functional integrity, it can still be observed that constructing clinically relevant vascular and vascularized tissue constructs are still far in the future. Innovative inventions enhancing the resolution and obtaining more anatomically accurate constructs while allowing scaling up of bioprinted tissues are required to provide potential solutions. Progressively, it may be expected that independent research on materials and cellular bioink materials for vascularization may cede place to more integrated and holistic approaches for developing bioprinters for vascularized tissue synthesis.



2019-1-BG01-KA203-062371

10. Other tissue types

Additionally, to the described tissue types, some scientists are also working in bioprinting of retinal and brain tissues, although these studies are still at their early fundamental level. A piezoelectric inkjet bioprinting was applied using retinal ganglion cells (RGCs) and glia and the effect of bioprinting parameters on the viability of cells and their growth-promoting properties were investigated. Recently, manual deposition of primary cortical neuron-laden gellan-gum-RGD for brain-like tissue fabrication was also presented. Although no computer control motion system was employed, the presented work demonstrated for the first time the use of layer-by-layer approach for brain tissue engineering.

TRANSPLANTATION AND CLINICS

Regenerative medicine is probably one of the most thrilling future applications of 3D bioprinting. Scientists hope that bioprinting can be used to directly print tissues such as skin, and to create organs, tissues, and structures for transplantation. 3D bioprinting is believed to be successfully applied for the transplantation and production of multilayered tissue types like nerve, cardiac, blood vessel, bone, and skin (Fig. 2). Researchers anticipate that in the future tissues and eventually whole organs could be transplanted to patients produced from their own cells. With the help of 3D bioprinting, transplant rejection and long donor lists could become an issue of the past. In addition, numerous tissue constructs were bioprinted and implanted subcutaneously to evaluate *in vivo* differentiation of cells and functionality of implants. Despite these experiments, none of the bioprinted tissues has been clinically used for humans because no approval has been granted yet. There are no regulations laid down for bioprinters or bioprinted products. However, with the growing interest worldwide and emerging businesses in the bioprinting market, the success with the first technology going through regulations will be exemplary for preceding technologies and products.

Although bioprinting technology is still in its early stages in clinics, 3D-printed plastic, ceramic or metallic implants for bone tissue replacement have been already successfully transplanted into humans. In addition to permanent implants, a recent work published in New England Journal of Medicine presented a unique case of transplantation of a 3D-printed bioresorbable airway splint into an infant. No unexpected problems have been observed with the splint and full resorption of the device is anticipated to take around 3 years. This was an exemplary case for clinical use of 3D-printed scaffolds and optimistically will lead to similar success with bioprinted tissues and organs. Despite the achievements in bioprinting research, bioprinting for transplantation in a hospital requires further improvements and translational efforts. Organs and tissues that do not need significant vascularization (i.e. skin and cartilage) are expected to be clinically translated very soon. However, tissues and organs that are metabolically highly active (i.e. heart, pancreas and liver) are extremely challenging.

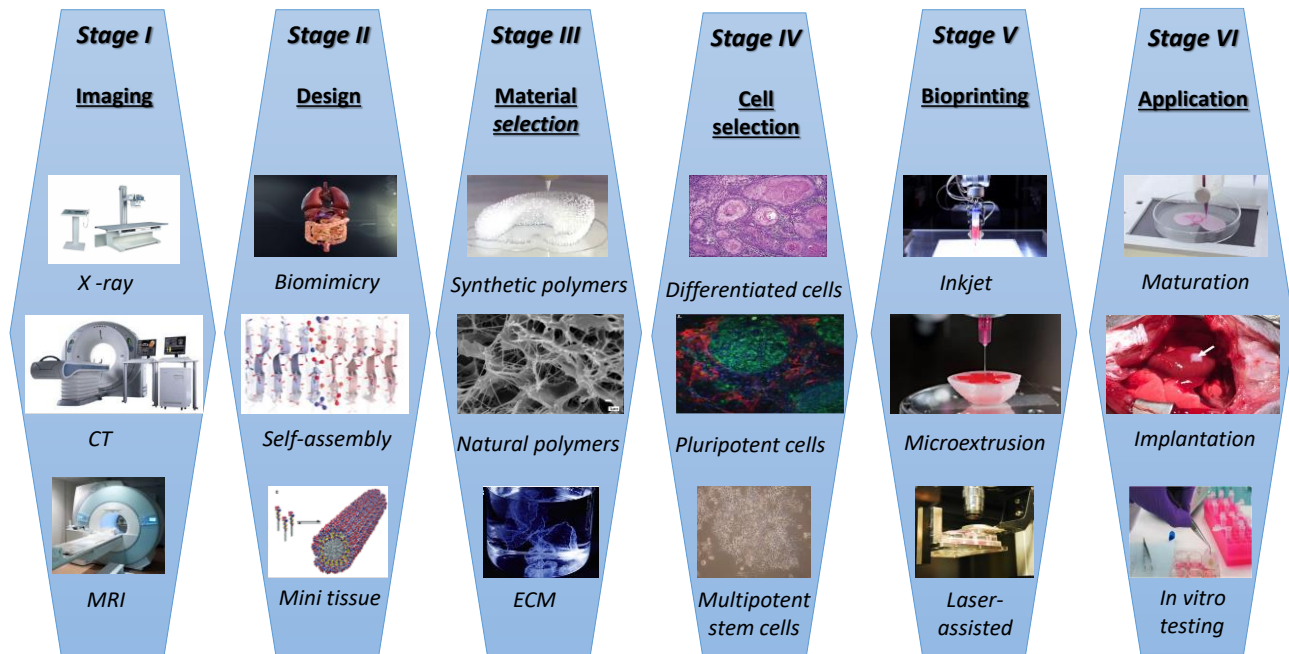


Figure 2. 3D printing strategy

One of the arguments for the clinical translation potential of bioprinted tissues and organs originates from the bioprinting technology itself. Bioprinting involves living cells in bioink and the use of patient-specific cells is relatively new in bioprinting. Stem cells, such as embryonic stem cells and induced-pluripotent stem cells, have been prospective unlimited sources of patient-specific cells for production of tissues and organs. Patient-specific cells can be differentiated and then bioprinted or bioprinted and then differentiated toward multiple lineages to construct tissues and organs that will cause minimum immunogenicity risk.

PHARMACEUTICS AND HIGH-THROUGHPUT SCREENING

Another key area where 3D bioprinting is expected to succeed is in the drug development. Most of the drug testing is currently carried out by using 2D cell cultures. Like cancer research, these cultures do not simulate natural cell behaviour. Xenografting (human cell transplantation) to animals is now employed to help solve this issue. However, this approach is challenging due to the biochemical, metabolic, and genetic differences between the species. It has been suggested that these issues are the main reason for the high rate of failure in assessment of the new drug candidates. Despite continuing efforts to improve the drug development process, only one out of 10,000 new chemical substances and one out of ten drug candidates enters clinical trials, reaches the final approval stage, and enters the

market. On the other hand, the use of 3D bioprinting for pharmaceutical testing rather than *in vivo* studies does not need regulatory approval. Thus, the throughput of drug development will possibly be extensively multiplied. Among different methods for engineering 3D *in vitro* systems, bioprinting has serious advantages such as controllability on size and microarchitecture, high-throughput capacity, co-culture opportunities and low-risk of cross-contamination. That is why, the bioprinted tissues and organs have been increasingly considered as potential models to be used in drug toxicology and high-throughput screening.

Liver and tumour tissue have been a focus in construction of tissue models for pharmaceuticals. In this respect the fabrication of a bioprinted liver micro-organ model for drug metabolism has been investigated (Fig. 3). The bioprinted 3D liver micro-organ has been used for simulation of the physiological liver response to drug administrations and toxic chemical exposure. The drug metabolism was demonstrated by the transformation of the nonfluorescent pro-drug, 7-ethoxy-4-trifluoromethyl coumarin (EFC), to an effluent fluorescent metabolite 7-hydroxy-4-trifluoromethyl coumarin (HFC). Epithelial cells and hepatocytes encapsulated in Matrigel™ were utilized to show the path of drug diffusing from the bloodstream to the tissue. Epithelial cells are the cells covering the lumen through which the drugs pass from bloodstream to the target, where hepatocytes were used as the target cell type. Based on the presented tissue model, a computational macroscale model was developed using a convection-diffusion-cell kinetics numerical framework. Latest is proven to be very helpful for future research in 3D micro-organ pharmacokinetics and toxicity.

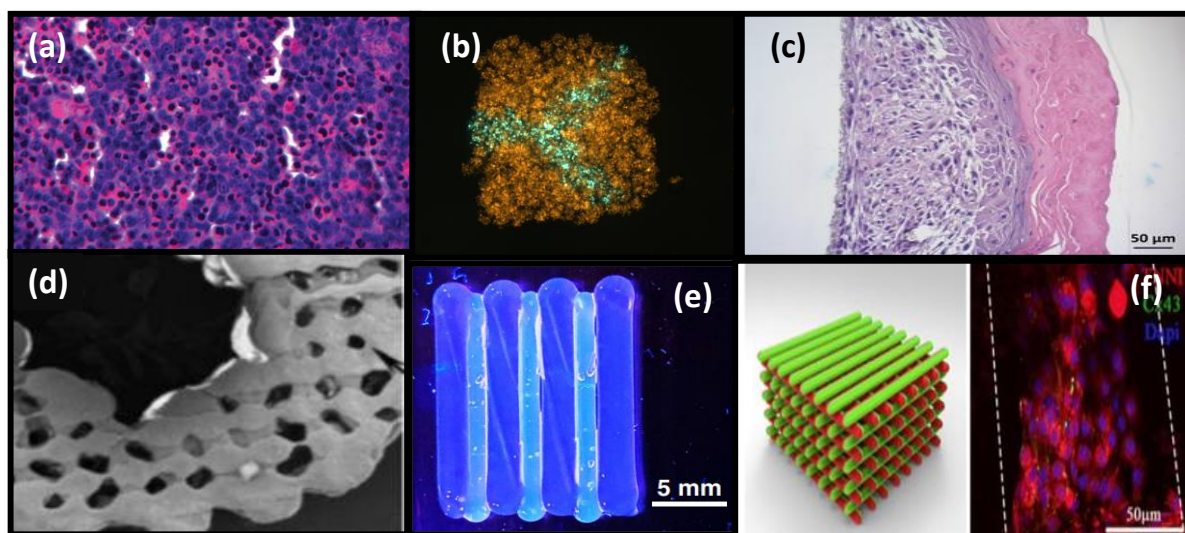


Figure 3. Bioprinted tissue models for drug testing and high-throughput screening. (a) Bioprinted liver tissue. (b) bioprinted kidney tissue (c) bioprinted skin tissue (d) 3D bioprinted bone repair material (e) printed cell-containing hydrogel (f) multicellular 3D bioprinted cardiac tissue constructs



2019-1-BG01-KA203-062371

Evolving microengineering technologies allow adaptable production of 3D cell-based microarrays including soft lithography, surface patterning, microfluidic-based manipulation and bioprinting. Among them, bioprinting technology has the advantage of creating co-culture and single-cell array with precise control on cell density. A recent study utilized a novel inkjet-based bioprinting method for fabricating a high-throughput miniature drug-screening platform. The authors bioprinted *Escherichia coli*-laden alginate to array a chip on coverslips. Using layer-by-layer approach droplets of three antibiotics were printed on the spots of cells. Results showed similar cell viability, functionality and antibacterial effects of antibiotics in inkjet bioprinted and micropipetted samples. Thus, the inkjet-bioprinted high-throughput arrays is proven to be effective method to minimize the duration of typical drug screening tests. Furthermore, cell-based biosensors (CBBs) were presented, where acoustic-based bioprinting was used to create a microarray of smooth muscle cell droplets in collagen. The bioprinted microarray was then subjected to different environmental conditions (e.g. temperature) and the effect of the applied stimuli as well as the bioprinting process parameters on cell viability was assessed. In addition to 3D bioprinting of high-throughput cell microarrays, controlled delivery of drug candidates into cell microarrays is also an important factor for effective drug screening. Different methods have been proposed for controlled drug delivery onto cell microarrays, including drug patterning, drug stamping, aerosol sprays and microfluidic drug loading.

CANCER RESEARCH

The immense future possibilities of 3D bioprinting technology in biomedical field has already been well documented. One of the areas where scientists are hoping 3D bioprinting to prove beneficial is the cancer research. The tumour microenvironment, as well as the biological structures and substances surrounding the tumour is an essential elements of cancer behaviour and is not yet fully understood. Up to now animal models and 2D cell cultures have been employed in these investigations. However, they do not precisely demonstrate the behaviour of human cancer cells *in vivo*, which includes a complicated interaction between tumour cells with both the cellular environment and other cells. Latest research suggest that 3D models more precisely reflect tumour cell behaviour and bioprinting is a promising technique that could be effective in such studies. One advantage that 3D bioprinting possesses is that cells can be explicitly designed into a 3D model with its extracellular and structural elements. Alternative approaches differ in that they require self-assembly, which is a much more time-consuming, or scaffolding, where the support matrix is developed independently from the cell culture. Furthermore, 3D bioprinting provide both the opportunity to print particular tissue and cell structures and also to be specific to patient. The cells of a patient can be biopsied, grown, and bioprinted, appropriate structures to be fabricated, and drug response or effective dose to be investigated.

However, use of bioprinting approach in cancer research is new and only a few studies have been performed in this emerging application area. For the first time the bioprinting of tumour tissue was demonstrated with human ovarian cancer (OVCAR-5) cells and MRC-5 fibroblasts. In this study an inkjet-based bioprinting platform with dual-ejectors was used. The proposed approach did not only show



2019-1-BG01-KA203-062371

a tool for cancer research but also offered a great platform for high-throughput screening. Same researchers performed bioprinting of HeLa cells to form cervical tumour models. Although these two studies presented bioproduction of tumour spheroids using bioprinting technology, construction of larger tissue models to examine cancer cell migration and metastasis is also crucial. Other approach was concentrated on the use of a laser-based 3D projection printing system to bioprint HeLa cells and noncancerous 10 T1/2 fibroblasts in PEGDA along with microvascular network with channel widths of 25, 45 and 120 μm to simulate blood vessel diameters. In addition to scaffold-based approaches, scaffold-free bioprinting of a breast cancer model using the NovoGen Bioprinting™ platform was also performed. In this attempt cancer cells were surrounded by a physiologically relevant stromal medium comprising MSC-differentiated adipose cells, mammary fibroblasts and endothelial cells.

CONCLUDING REMARKS AND FUTURE OUTLOOK

Recent investigations have demonstrated that 3D bioprinting is an emerging approach having broad application in various areas including basic research in tissue engineering, regenerative medicine and cancer pathogenesis, tissue bioprinting for transplantation and clinics, and could be successfully applied in pharmaceuticals for drug testing and high-throughput screening. Currently, there are around 15 different tissue types used in different experimental studies. However, there still exist several other human tissues that have not been touched yet. In this regard, future attempts must be made in bioprinting of other tissue and organ models that are challenging but can revolutionize medicine. This also depends on the innovations in the field of tissue engineering as bioprinting research vitally depends on our understanding of unexplored tissue types. In addition, one of the new directions in bioprinting should be focused on biofabrication of new types of organs, such as bionic organs or organs that do not exist in nature.

The majority of 3D bioprinting research has developed around homocellular tissue models using a single cell type. However, native tissues have a heterocellular nature with multiple cell types organised in highly complex anatomy. Although simple bioprinting tissue models are relatively acceptable in fundamental research, fabrication of functional tissue for clinical or pharmaceutical studies requires inclusion of multiple cell types. Thus, some of the functionality of cells can be enabled or further boosted by cell–cell interactions. Therefore, bioprinting multiple cell types needs further investigations of the optimum culture conditions including the right medium and reagents to support their growth and behaviour.

Contemporary attempts in translation of bioprinting to transplantation are mainly limited to murine models. However, murine models are small with physiological conditions significantly differ from those of humans. For example, a small pancreatic tissue with 3D bioprinted islets can be implanted into a diabetic murine model and can regulate insulin secretion. On the other hand, the average volume of islets in a mouse pancreas is approximately 100,000 times smaller than those in a human pancreas. Therefore, bioprinting of scale-up tissues and organs is extremely important to construct them at



2019-1-BG01-KA203-062371

clinically relevant dimensions. Larger animals such as a porcine model can be a transitional step toward trials on humans because these animals correspond to human physiology more closely than small animals. To scale-up bioprinted organs for clinical use, fabrication of hierarchical vascular networks is crucial in addition to their mechanical strength, elasticity, and long-term structural stability. Additionally, integration of nerve tissues and forming innervation is a critical step toward functional tissue and organ production because tissues such as cardiac, muscle and skin require innervation, which is currently a challenge in bioprinting. The majority of bioprinting research involves the use of hydrogel-based bioinks as they are favourable environment for 3D growth of cells. However, hydrogels have weak mechanical properties when they are used at low concentrations to support cell proliferation. In addition, biodegradable materials such as thermoplastics can be utilized as a background frame for the scale-up of tissues and organs. Available biomaterials, such as synthetic polymers, are strong, but their degradation is difficult, time-consuming, and does not match with the tissue regeneration process closely. Therefore, new biomaterial development is vital for the progression in bioprinting of tissues and organs for clinical use. Apart from the biomaterials, selection of the right cell source is also a critical factor for successful clinical and pharmaceutical use. For personalized medicine it is challenging to obtain different primary cell types. Stem cells represent a promising source of cells and further investigations are needed to establish standard protocols for differentiation of stem cells into various stable and functional cell lineages.

Another prominent way of tissue fabrication for high-throughput screening are the miniature 3D-array platforms such as tissue-on-a-chip or organ-on-a-chip models. Blend of bioprinting technology with other technologies such as microfluidics is essential for producing physiologically simulated, complex and long-term viable target micro-organ arrays. Application of technologies generating picoliter droplets with low level of cell damage and having single cell sorting capabilities should be encouraged in bioprinting of cell microarrays. Controlled drug delivery onto cell microarrays should be also considered and implemented.

Although several attempts are made in engineering cancer microenvironment using bioprinting technology, further advancements are needed for construction of a physiologically relevant complete microenvironment, comprising tumour site, healthy site, and microvascular network in between, to perform cancer metastasis research. Tumour tissue models should be precisely placed within bioprinted vascularized parenchymal tissues to study their growth, tumour cell intravasation and extravasation.



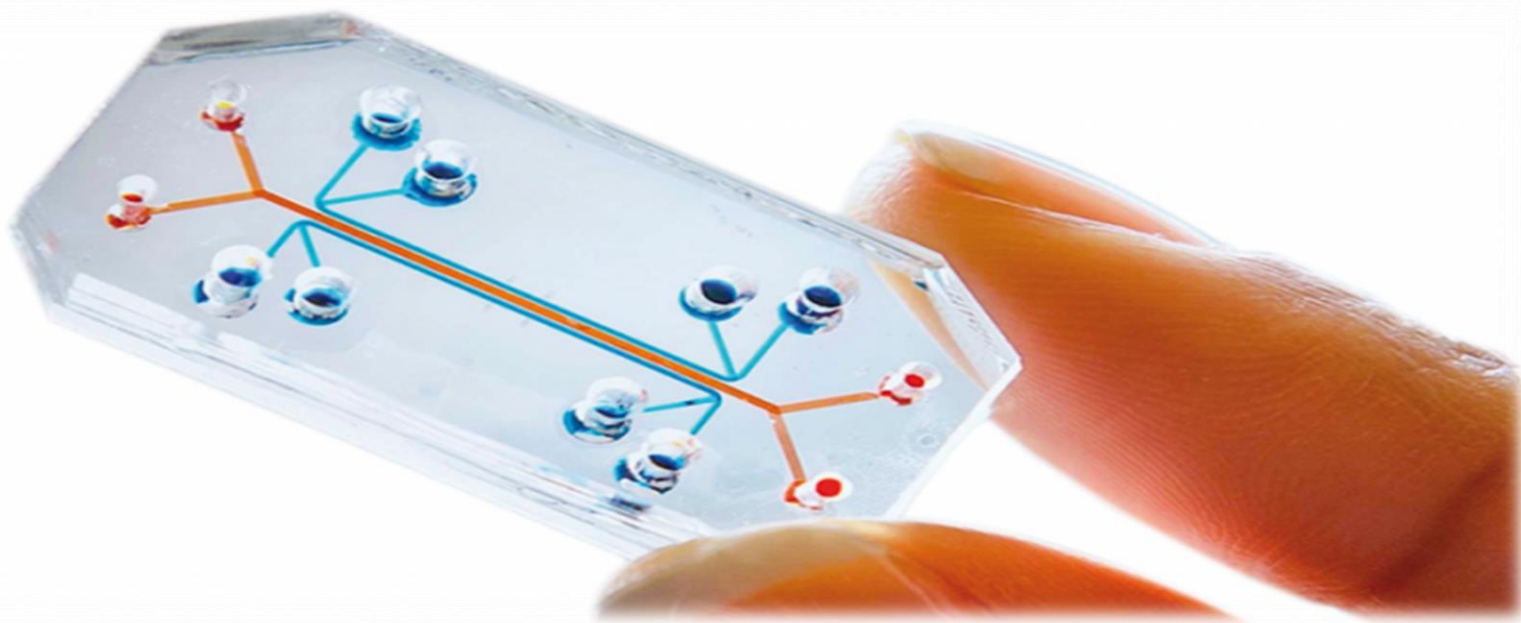
References

- AZoMaterials. 3D bioprinting and its applications (2018).
<https://www.azom.com/article.aspx?ArticleID=17370>
- Bhise NS et al. 2014. Organ-on-a-chip platforms for studying drug delivery systems. *Journal of Controlled Release*. doi:10.1016/j.jconrel.2014.05.004
- Harris M. 2018. Print me an organ. <https://physicsworld.com/a/print-me-an-organ/>
- Liang M, Yutong W, Li Y, et al. 2020. Current Advances on 3D-Bioprinted Liver Tissue Models. *Adv. Healthcare Mat.*, doi: 10.1002/adhm.202001517
- Murphy SV, Atala A. 2014. 3D bioprinting of tissues and organs. *Nat. Biotechnol.*, 32: 773–785.
- Varotto A. 2015. *Global Market for 3D Printing.*, BCC Research, ISBN: 1-62296 -006-8
- Ozbolat IT, Peng W, Ozbolat V. 2016. Application areas of 3D bioprinting. *Drug Discovery Today*. doi:10.1016/j.drudis.2016.04.006
- Park JY, Jang J, Kang HW. 2018. 3D Bioprinting and its application to organ-on-a-chip. *Microelectron. Eng.*. doi:10.1016/j.mee.2018.08.004
- Wang C, et al. 2014. Three-dimensional *in vitro* cancer models: a short review. *Biofabrication*, doi: 10.1088/1758-5082/6/2/022001.
- Yao R, et al. 2016. Three-dimensional printing: review of application in medicine and hepatic surgery. *Cancer Biol Med*
- Grigoryan B, Paulsen S, Corbett D, et al. 2019. Functional intravascular topologies and multivascular networks within biocompatible hydrogels. *Science*, 464: 458-464.
- Augustine R. 2018. Skin bioprinting: a novel approach for creating artificial skin from synthetic and natural building blocks. *Prog Biomater.*, 7: 77–92.
- Datta P, Ayanb B, Ozbolat IT. 2017. Bioprinting for vascular and vascularized tissue biofabrication. *Acta Biomaterialia*, 51: 1-20.



Project website: www.digit-biotech.eu

The European Commission's support for the production of this publication does not constitute an endorsement of the contents, which reflect the views only of the authors, and the Commission cannot be held responsible for any use which may be made of the information contained therein.



LO3 BIOSENSORS & BIOCHIPS FOR SUSTAINABLE FUTURE

Advanced level

AUTHOR:
TSVETINA MIHAILOVA & KLIMENT PETROV



Contents

Biosensors & Biochips Technologies: Contribution to the Future Sustainable Life.....	3
Biosensors & biochips: advances in medical diagnostics	4
1. Glucose biosensors in diabetic management.....	6
2. Cardiovascular disease detection using biosensors	7
3. Biosensor for detection of cancer.....	7
4. Biochip in diagnostics	8
5. Biochip in Tuberculosis epidemic.....	8
6. Biochip in cancer.....	9
Biosensors & Biochips applied in food and agriculture.....	9
1. Nanomaterials in biosensing technology.....	12
2. Nutrient and quality detection	12
3. Detection of pathogens.....	13
4. Detection of toxins	14
Biosensors & Biochips for environmental monitoring.....	16
1. Pesticides.....	17
2. Pathogens	18
3. Potentially toxic elements	19
4. Toxins.....	20
5. Endocrine disrupting chemicals	20
6. Other environmental compounds	21
References	21



Biosensors & Biochips Technologies: Contribution to the Future Sustainable Life

The field of synthetic biology has exploded over the past decade, having a major influence on fields such as metabolic engineering, protein engineering, digital biology, and whole-genome engineering. In the framework of iterative "design-build-test" development cycles, a significant portion of synthetic biology innovation has taken place. In the field of synthetic biology, progress can be associated with innovations in each of the processes of "design", "build" and "test". For example, there has been a major push to standardize elements within synthetic biology, with significant attention being paid to modularity and "plug and play" components. This modularization, along with the accelerated progress in systems biology, has allowed the "design" stage to become less time-consuming and less reliant on advanced knowledge. In recent years, the cost of DNA sequencing and synthesis has also decreased dramatically, allowing large constructs to be synthesized cheaply. In the 'build' phase, this has facilitated a rapid improvement, helping researchers to investigate a larger percentage of the space of the biological solution. Finally, within the synthetic biology "test" phase, high-throughput screening has also become a focal point. The increased "design" and "build" potential has contributed to an increased demand for success in the assessment of the plethora of new designs. In turn, this was done by incorporating robots and high-throughput analytics into the laboratory setting, in which new models can be evaluated to a level that is not achievable for human researchers.

Biosensors represent a groundbreaking emerging technology for high-throughput screening that can be implemented. Most precisely, they are classified as an analytical tool consisting of biological components used to detect and generate a signal for the presence of a target ligand. Synthetic biology is at the forefront of biosensors, both as a tool for high-throughput screening, but also as the direct result of developments within the field of synthetic biology itself. In addition, because of the unparalleled specificity and sensitivity that biological parts provide relative to conventional analytical methods, biosensors have gained expanded interest as alternatives to traditional analytics.

The design and construction of biosensors is a multidisciplinary endeavor and can include expertise in areas such as protein engineering, molecular biology, affinity chemistry, molecular dynamics of nucleic acid, materials sciences, and nanotechnology. Biosensors interface with a target ligand at their most simple stage, undergo some type of modification, and output a signal. There is a great variety of potential configurations in all the parts of this process. Target ligands range from single atoms such as calcium, to entire proteins such as thrombin, all the way through. Processes as varied as enzymatic activity, fluorescence, electrical current generation, and transcriptional activity include output signals. The mechanisms that transduce ligand recognition into functional signals are just as diverse.



2019-1-BG01-KA203-062371

In the field of analytics, biosensors represent a significant step forward. In order to move analytics away from purely physics- or chemistry-based frameworks, the integration of biological components in sensory diagnostics has begun. This has allowed analytical functions that are not well adapted to conventional methods to conduct a vast diversity and specificity of biological components. The theoretical and demonstrated biosensor applications cover a significant range of human society and activity. Biosensor applications are grouped into three broad categories, depending on their measurement scale.

- ❖ Group Diagnostics: Environmental, Agricultural, and Industrial Applications
- ❖ Point-of-Use Diagnostics: Medical, and Security Applications
- ❖ Single-Cell Diagnostics: Metabolic Engineering, and Synthetic Biology Applications

BIOSENSORS & BIOCHIPS: ADVANCES IN MEDICAL DIAGNOSTICS

Biosensors consist of a biocatalyst that can recognize a biological element and a transducer that can turn the biocatalyst and the biological element combination occurrence into a measurable parameter.

The biocatalyst may be biomolecules such as enzymes, DNA, RNA, metabolites, cells, oligonucleotides, etc., and electrochemical, calorimetric, optical, acoustic, piezoelectric, etc. transducers. Biosensors using immobilized cells, enzymes and nucleic acids have come into the field in recent years in disease diagnostics. For engineering disease diagnostic biosensors, nanobiosensors utilizing the ultra-small size and unique properties have also been applied. The use of biosensors can quickly determine the health status, the onset and progression of the disease and, with the assistance of a multidisciplinary combination of chemistry, medical science and nanotechnology, can help to prepare treatment for many diseases. The devices are cost-effective, highly responsive, fast, user-friendly, and can be manufactured for human use in bulk. Numerous biosensors for the diagnosis of three major diseases, such as diabetes, cardiovascular disease and cancer, are the most developed ones.

Such biosensors, coined by Cammann, are analytical instruments that transform an electrical signal into a biological response. Biosensors can usually be highly precise and should be recyclable and irrespective of physical limitations such as pH, temperature. Practical approach to the design of a biosensor requires manufacturing, immobilization, transduction devices that offer multidisciplinary research engineering in both chemistry and biology.

Based on their working mechanism the diagnostic biosensors are divided into four major groups:

1. Enzyme-based biocatalytic biosensors.
2. Bioaffinity group, i.e. antibody, antigen and nucleic acid presence.
3. Microbes, i.e., microorganism-containing biosensors.
4. Nanosensors, i.e. active nanoparticle sensors that typically increase sensitivity and specificity for early disease detection.

2019-1-BG01-KA203-062371

These various types of biosensors help hormone levels, drugs, toxins, contaminants, heavy metals, pesticides, etc. to be identified with significant specificity.

Biosensors are tools that commonly estimate biological marker levels or any chemical reaction by creating signals that are primarily associated with an analyte's concentration in the chemical reaction. Typically, such biosensors help monitor diseases, drug discovery, pollutant detection, bacteria-causing disease detection, and markers that usually indicate diseased conditions, such as body fluids (saliva, blood, urine, sweat, etc.). A typical biosensor is shown in Figure 1.

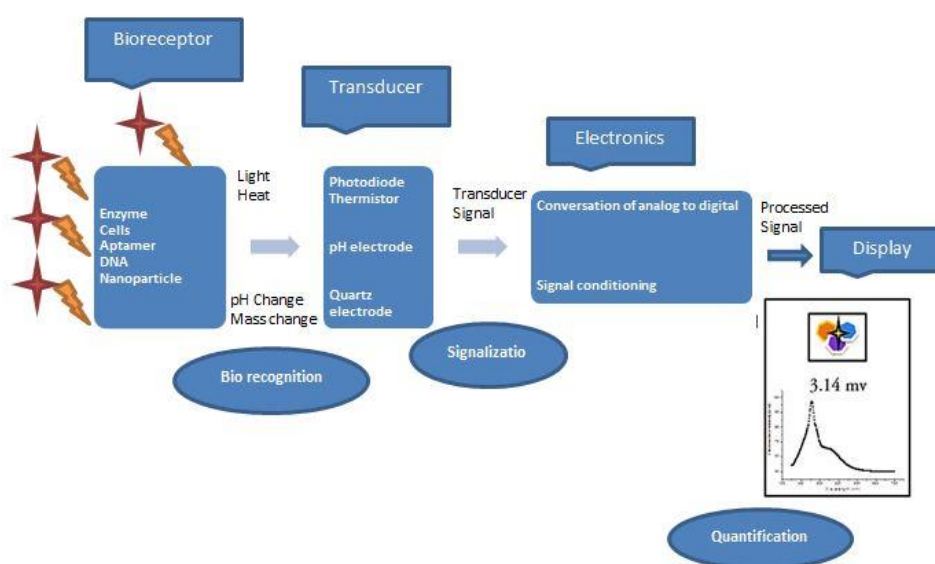


Figure 1. Schematic depiction of biosensor

A typical biosensor is composed of:

1. Analyte: A substance of interest, such as glucose for diabetes, that needs to be established.
2. Bioreceptor: A bioreceptor for enzymes may be a molecule which recognizes the analyte.
3. Transducer: Normally, a bio recognition event is converted into a detectable signal, known as signalization.
4. Electronics: In display form, it typically processes the transduced signal.
5. Display: Typically, the liquid crystal display results in a user-friendly manner in combination with hardware and software for biosensor generation.

There are several biosensor applications that have been introduced in different areas, such as medical science, the marine sector, the food industry, etc., and these biosensors are often programmed for improved sensitivity and linearity compared to conventional methods. However, the application of biosensors is growing increasingly in the field of medical science.

1. Glucose biosensors in diabetic management

Blood glucose monitoring has become a valuable tool in the management of diabetes and daily blood glucose levels are typically maintained by consulting clinicians who have developed a series of blood glucose sensors. *Diabetes mellitus* is the largest prevailing carbohydrate metabolism endocrine disorder with more morbidity and mortality in developing countries. Multiple tests are usual in diabetic patients for the investigation and monitoring of diabetic markers. The key diagnosis criteria for diabetes are the level of blood glucose, which includes diabetic patients' self-monitoring of glucose levels. Studies have shown that microvascular (nephropathy, neuropathy, and retinopathy) and macrovascular (coronary artery disease and stroke) complications can be improved by controlling the level of blood glucose in the normal range. Blood glucose is typically observed in healthy individuals in the range of 4.9-6.9 mM and can increase in diabetic patients up to 40 mM after glucose intake. Although different kinds of glucose sensors are commercially available, the third generation of glucose biosensors is shown in Figure 2 as an example.

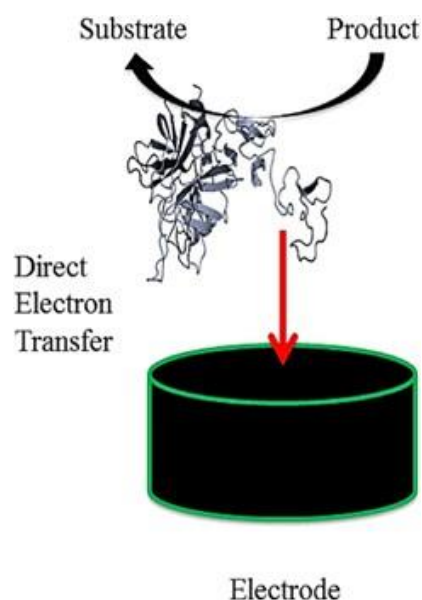


Figure 2. Third generation of glucose biosensor



2019-1-BG01-KA203-062371

2. Cardiovascular disease detection using biosensors

The number of deaths caused globally by cardiovascular disease (CVD) is significant and more people die of CVD than by any other disease. By 2015, about 17.7 million people had died from CVD, representing a total of 31% of all global deaths. 7.4 million of these were due to coronary heart disease and 6.7 million were due to stroke. By way of medication and therapy, a person with CVD needs earlier detection and management. The current CVD detection strategy relies on the traditional method, which is usually based on testing that can take many hours or even days. The WHO sets these diagnostic criteria, under which patients should follow at least one of the conditions, such as changes in the diagnostic electrocardiogram (ECG), elevation of biochemical markers in their blood samples, and characteristic chest pain. ECG is an important parameter for therapy management, but ECG is a poor diagnostic test in the case of CVD because half of CVD patients have a normal cardiogram, making it more difficult to diagnose this medical condition. Biosensor will aid in rapid diagnosis, providing excellent health care and reducing the delay time for the distribution of the results, which is immense stress for the patients.

3. Biosensor for detection of cancer

Cancer is one of the most lethal diseases, and several researchers have recently developed biosensors for early cancer detection. Most cancers are typically diagnosed by MRI, ultrasound or biopsy methods that rely on the physical properties and presence of the tumor and identify either advanced or invasive instruments. The variations in gene sequences, i.e. mutations, primarily cause cancer and thus require early diagnosis before the disease progresses. Early cancer detection makes treatment faster and more successful, opening up a biosensor platform for the detection of early cancer stages. Many experts assume that in the case of cancer, early detection could be possible because abnormalities in chemical and genetic composition may be identified long before the disease begins. Uncontrolled and irregular cell growth, commonly believed to be cancer, occurs due to the accumulation of unique genetic mutations and epigenetic defects. The tumor cells are shown to be resistant to apoptosis and the body's anti-growth defense mechanism. If it progresses and begins to expand to other body organs and systems, i.e. metastasize stage, the cancer becomes incurable. Oncogene stimulation and reducing the function of tumor suppressor genes (TSGs) are the two most important tumorigenesis mechanisms. Due to mutation or replication of normal gene (proto-oncogene), activation of oncogene takes place, which plays key roles including, control of cell growth, proliferation, and/or differentiation. Such genetic mutation guides the gene to produce an excess quantity of its gene product, resulting in dysregulation of cell division, cell growth and tumor establishment. Many oncogenes have been considered as promising cancer biomarkers for growth factor receptors. In ~ 33% of all breast cancers, the human epidermal growth factor receptor Her-2 is intensified, and cancers with strengthened Her-2 seem to develop and increase more rapidly. Awareness of Her-2 status is therefore essential in concluding the possible medication course. Trastuzumab is now a typical adjuvant therapy for patients with this type of amplified gene expression, a recombinant humanized monoclonal antibody targeted at Her-2 as a straight-forward treatment for breast cancer. TSGs are related to the control of insufficient cell growth and proliferation by minimizing



2019-1-BG01-KA203-062371

or preventing the division of cells. Retinoblastoma protein (Rb), BRCA1/2, and p53 are three of the well-studied TSGs in cancer. Rb is a master cell division regulator, and Rb mutation plays a significant role in various cancers. The most common causes of inactivation of the Rb1 gene are point mutations and deletions. BRCA1 is a DNA repair enzyme that is associated with newly replicated DNA 'proofreading' for fidelity and to search for any mutations. Until the cell divides, DNA repair enzymes normally work to excise replication errors. BRCA1 gene mutations are responsible for 50% of hereditary breast cancers and 80-90% of hereditary breast and ovarian cancers. Lastly, a main regulator of apoptosis or programmed cell death is the p53 protein. In the brain, breast, colon, lung, hepatocellular carcinomas, and leukaemia, p53 mutations are found. Another significant involvement with p53 loss is that it leads to the mechanism of resistance of chemotherapy drugs. The improvement of biosensors that can detect the existence of p53, Rb, and BRCA1 mutations is highly warranted and can enable us to evaluate the susceptibility of early cancer with detailed prognosis and treatment regimes.

4. Biochip in diagnostics

The DNA biochip opens up a new genetics-based field of diagnostics. The way the medical profession performs blood testing could be revolutionized by a newly developed DNA biochips. They are virtually immediate with the matchbox-sized biochip instead of a patient having to wait several days for results from a laboratory. And with no sacrifice of accuracy, it requires less blood. The DNA biochip reduces the need for radioactive labels used for detection, in addition to time savings. For technicians and laboratory workers handling samples and performing tests, this significantly decreases costs and future health effects. It also lowers disposal costs because, according to strict regulations, chemically labelled blood must be handled.

A biosensor must be highly sensitive and able to differentiate between, for example, bacteria, viruses or other chemical or biological species to be useful for detecting compounds in a real-life sample. According to Vo-Dinh, who clarified that the biochip mimics the sophisticated recognition capabilities of a living system, DNA biochips do that. The DNA biochip is a gene probe-based biosensor, as opposed to other biosensors based on enzyme and antibody probes. Gene probe-based biosensors provide exceptional selectivity and sensitivity, making them valuable tools for diagnosing genetic diseases and infectious species.

5. Biochip in Tuberculosis epidemic

The development of new biochip technologies by Russian and American scientists could bring some hope of halting the global resurgence of tuberculosis. Established by the U.S. Department of Energy's Argonne National Laboratory and the Russian Academy of Sciences' W. A. Engelhardt Institute



2019-1-BG01-KA203-062371

of Molecular Biology (Moscow), the technology is intended to help combat the current variety of drug-resistant strains of the disease.

The World Health Organization reports that tuberculosis kills more young people and adults, including AIDS and malaria combined, than any other infectious disease. The biggest challenge of the ongoing tuberculosis epidemic is that the disease can be caused by several different bacterial species, and each one is resistant to various drugs. The critical element in controlling the disease is to define the strain that affects a given patient and to determine the best antibiotic for combating that strain. To differentiate between numerous tuberculosis strains, Argonne intends to use biochip technology in research. Testing on segments of genetic material removed from tuberculosis bacteria would initially be carried out. Biochips are designed to simultaneously conduct a number of biochemical reactions and have been found to perform satisfactorily in laboratory testing. Since the detection of specific tuberculosis strains takes weeks or months, patients are frequently prescribed several antibiotics simultaneously.

6. Biochip in cancer

The biosensor chip technology also provides fast and simple access to crucial information about cancer-producing compound DNA damage, moving researchers a step closer in the fight against cancer. Unlike traditional methods of biosensing, a laser-based, high-resolution and low-temperature fluorescence method offers a precise fingerprint of the molecule. It is possible that its ease of use encourages the replacement of invasive endoscopic procedures and helps to detect colon cancer early on.

BIOSENSORS & BIOCHIPS APPLIED IN FOOD AND AGRICULTURE

The current food production faces immense challenges from the increasing human population, the maintenance of clean resources and food quality, and the protection of the environment and climate. Food sustainability is mainly a cooperative effort that results in the development of technology funded by both governments and companies. Several attempts have been supported to overcome challenges and improve the drivers in food production. Via their applications, biosensors and biosensing technologies are widely used to solve the major challenges of food production and its sustainability. As a result, there is a rising need for biosensing technology in the area of food sustainability. A technological system combining several technologies is defined by microfluidics. Nanomaterials, with its biosensing technology, is known to be the most innovative tool strongly associated with world populations in dealing with health, energy, and environmental issues. The need for point of care (POC) technology in this area focuses on analytical tools that are fast, simple, precise, compact, and low-cost.



2019-1-BG01-KA203-062371

For our existence and lives, food with its production industry is essential; and its sustainability is essential in continuous human growth on the planet. Current food production is facing immense difficulties from increasing human population, maintaining clean resources and food quality, and protecting environment and climate. Some of these issues stem from food production itself; others stem from other food production-related industries. Food recalls, for example, trigger major damage to food brands' credibility and prestige, with an estimate of \$15 million per incident over the last few years. 48 million sick cases are responsible for 3000 fatalities annually due to foodborne illnesses.

Food safety is largely a cooperative effort arising from both governments and companies in technology development. In order to pose new challenges in food safety issues, information technologies such as blockchain technology can accelerate communication between food quality, media and consumers. Five challenges can be summarized as the main challenges in the sustainability of food production: the production challenge of food safety and security; the quality challenge of food diversity and quality; the economic challenge in the leading food system, including its packaging and supply chain; the environmental challenge, including the processing of food waste; and the engineering challenge in the creation and generation of novel food.

Basically, a biosensor is an analytical instrument used to measure a sample's molecule of interest (target). In general, a bio-recognition factor (aptamer, antibody, enzyme, etc.) that is unique to the target is used. A physiochemical or biological signal is elicited by molecular recognition events between the recognition element and the target compound, which is transformed into a measurable quantity by the transducer. Signals are shown in either optical (colorimetric, fluorescence, chemiluminescence and plasmon surface resonance) or electrical (voltammetry, impedance and capacitance) or any other chosen format (Figure 3).

2019-1-BG01-KA203-062371

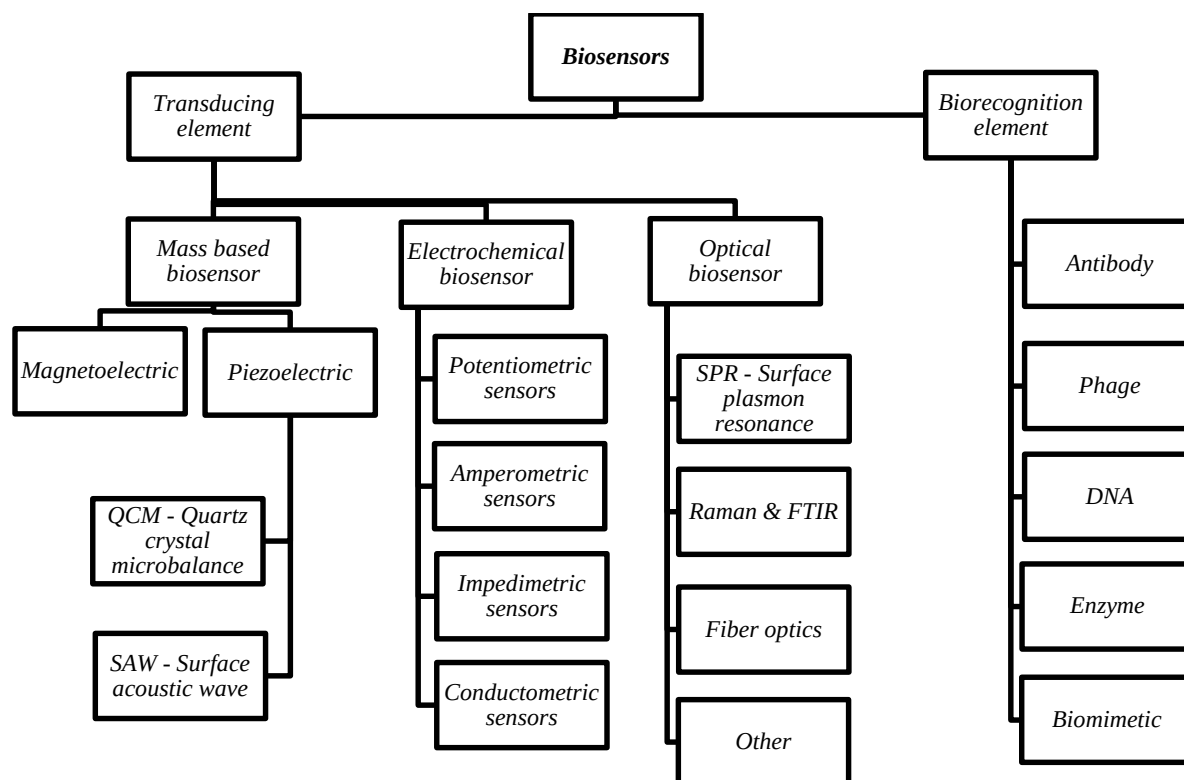


Figure 3. Classification of biosensors based on transducer and bio-recognition elements used in food analysis

As one of the primary objectives of food analysis, food safety is a major health issue in both animal and human lives. The advancement of food safety analytical technology means that it thrives in line with the rising interest in and emphasis on food supply safety issues. In food safety analysis, traditional approaches are labour-intensive, time-consuming, and need trained technicians. The application of microfluidics in food safety analysis provides fresh insight about how to detect foodborne toxins, allergens, pathogens, hazardous substances, heavy metals, and other contaminants effectively and rapidly. Microfluidics' features, such as its miniaturization capability, compact and reducible quantities of samples and reagents, make it a perfect technology for the development of food sustainability. Complex food matrix preparation and difficult manufacturing steps are the current challenges in the application of microfluidics to food sustainability. These challenges can be addressed by leveraging physical properties dependent on specific test targets, designing complex real food analysis microfluidic platforms, and incorporating into microfluidic systems biomolecules such as food proteins and DNA.



2019-1-BG01-KA203-062371

1. Nanomaterials in biosensing technology

With its biosensing technology, nanomaterials are the most promising tool in dealing with health, energy and environmental problems associated with population in the world. Particles smaller than 100 nm in at least one size dimension are known as nanomaterials. These nanomaterials are biocomposite polymers based on metal, metal oxide and carbon, and different types of nanoparticles have been established, such as magnetic iron, aluminum, gold, silver, copper, silica, zinc, zinc oxide, cerium oxide and titanium dioxide nanoparticles, and single/multiple walled carbon nanotubes (CNTs). Nanotechnology and its agricultural development have been greatly extended in different fields. These fields include food production, crop protection, detection of pathogens and toxins, purification of water, food packaging, disposal of wastewater, and environmental remediation. Improving the productivity and performance of applications is the priority of these agricultural fields.

In the field of food safety and protection, biosensing technologies have been developed for nutrient and quality detection, detection of pathogens and detection of toxins, as listed below.

2. Nutrient and quality detection

Food protection measures can be split down into two categories: post-harvest loss and food biosecurity. Food biosecurity means food contamination and degradation, which is addressed in the later sections, by environmental, political, unfair economic gain, warfare, or exacting revenge. Post-harvest loss, on the other hand, suggests the nutrients and edible conditions in food that need to be maintained between the harvest period and the moment of consumption by technologies. Since time differs from minutes to years, in maintaining and reducing losses, technologies focusing on reducing post-harvest losses are important.

To maintain food quality and to avoid post-harvest losses, new technology such as biosensing can be used. Biosensors have been developed, for example, to detect and analyse quantities of sweeteners in foods that can be used to detect both natural and artificial sweeteners. Sweeteners are widely used in food production and processing, but they have recently been identified in humans as causing health problems. A multi-channel biosensor has been developed to use electro-physiological sensing from taste epithelia to detect and analyse both natural and artificial sweeteners. To detect long-term signals from sucrose, glucose, cyclamate, and saccharin, respectively, the signals are studied through spatiotemporal techniques. The biosensor can distinguish between different concentrations with dose-dependent increased responses of the taste epithelium from different sweeteners. It can also distinguish between two natural sweeteners: sucrose and glucose, with two signal patterns. For glucose, the detection range is 50-150 mM, and for saccharin, 5-15 mM.



2019-1-BG01-KA203-062371

3. Detection of pathogens

Due to their reduced format, biosensors targeting pathogen detection such as bacteria (Table 1) and fungi (Table 2) started more than two decades ago; one device to address multiple problems, and a multi-panel signal detection. The ligand motif is a crucial element in the biosensor design for pathogen detection since it determines the sensitivity and efficiency of the device. The aim is to establish a fast, specific, and sensitive platform to detect in food samples the presence or absence of pathogens. It has been discovered that there is no ideal ligand, and various ligands have different advantages. The combination of bioreceptors to detect a large variety of microbes in different samples poses current challenges in pathogen biosensor detection; new synthetic ligand designs such as aptamers, small molecules, and peptides; and the incorporation of different ligands into a portable device to achieve rapid, effective, and low-cost detection.

Table 1. Conditions for numbers of bacteria grown in milk

<i>Temperature °C</i>	<i>24 h</i>	<i>48 h</i>	<i>96 h</i>	<i>168 h</i>
0	2400	2100	1850	1400
4	2500	3600	218,000	4,200,000
8	3100	12,000	1,480,000	
10	11,600	540,000		
15	180,000	28,000,000		
30	1,400,000,000			

Table 2. Temperature and water activity requirements for fungal growth

<i>Species</i>	<i>Minimum</i>	<i>Optimum</i>	<i>Maximum</i>	<i>Minimum</i>	<i>Optimum</i>
<i>Aspergillus ruber</i>	5	24	38	0.72	0.93
<i>A. amstelodami</i>	10	30	42	0.70	0.94
<i>A. flavus</i>	12	35	45	0.80	0.99
<i>A. fumigatus</i>	12	40	52	0.83	0.99
<i>A. niger</i>	10	35	45	0.77	0.99
<i>Penicillium martensii</i>	5	24	32	0.90	0.99

4. Detection of toxins

The mainstream of development in food safety is electrochemical biosensors for rapid detection and assessment of food toxins. Numerous platforms have been developed to allow customized and individualized devices to meet particular environmental and organizational requirements and to reach the nM to fM detection limit levels. For example, to encourage unique binding profiles, bioreceptor arrays address individual electrodes functionalized with different bioreceptors with binding targets. In addition to electrochemical biosensing, toxin and chemical detection in food production have been applied to other biosensors such as optic and piezoelectric sensing (Figure 4). In order to sense toxins, fluorescent nanoparticles have been produced in foods and bodies, including on-surface, inter- and intra-cellular foods.

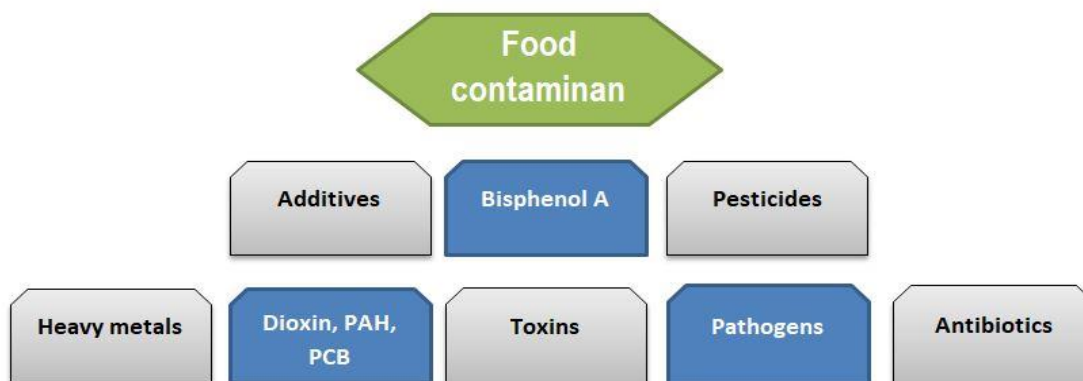


Figure 4. Predominant food contaminants and the target analytes in the food manufacturing industries

Toxin extraction from complicated food samples is one of the main obstacles in creating a fully automated toxin detector. To automatically assess their harmful levels from food and water samples, potential systems are expected to extract, process, and measure toxins. In identifying, discriminating, and quantifying chemical toxins in food matrices, sophisticated separation strategies have been coupled with SERS. In addition, even though they are typically in lower amounts, chemical contaminants from food processing can be a challenge. Lower stability, selectivity and sensitivity are another challenge in food toxin detection, where MIPs can be a solution to provide stable and low-cost alternatives.

Heavy metals like Ag^+ , As^{3+} , Cd^{2+} , Hg^{2+} , Pb^{2+} , and Zn^{2+} are known as chemical pollutants that form stable states of oxidation and interfere with metabolic pathways, resulting in health problems. Aptamer and DNA-based biosensors can detect heavy metals at both nanoscale and very large-scale levels, which are appropriate for food safety screening and monitoring. In order to detect arsenate in food, a heavy metal detecting biosensor is based on genetically modified bacterial cells and a green, fluorescent signal amplifier. With a detection range of 5-140 $\mu\text{g/L}$ of arsenic, its arsenic detection lasts just one hour and can be integrated with optical power output for its future biosensing optical fibre. Other biosensing technologies like aptamers, nanoparticles and graphene electrodes have been successfully applied to the identification and evaluation of arsenic, with the potential to be produced as fast, simple, easy-to-use, and low-cost devices.

Nanotechnology has been adapted to two separate fields of agri-food pesticides: as a pesticide delivery vector for pesticide management and as a trace-amount detector for pesticides. In the first field, nanoparticles are able to slowly modify pesticides to target insect pests, which helps prevent groundwater



2019-1-BG01-KA203-062371

and topsoil pollution, reduce pesticide levels and improve efficiency. In the second field, bio- or biomimetic-based nanotechnology, like antibodies, enzymes, aptamers, and MIP-like macromolecules, improves stability, selectivity, sensitivity, and speed of detection. Furthermore, bacterial, fungal, algal, and mammalian cells are all cell-based biosensors used in pesticide and herbicide detection, helping to establish fast, reliable, real-time, and cost-effective tools for decontamination procedures and preventive casualty damage.

Carcinogens, odorants, and marine contaminants are other toxins that are significant in food production. Carcinogens are a complex group of trace amount of toxins, like pesticides, heavy metals, mycotoxins, and acrylamide, in which the difficulty of identifying trace-amounts is a challenge; and imprinted aptamers, nanotechnology, and biosensing are optimistic for promising future use. Sensitive and soluble molecules effective in odour detection for olfactory animal systems are odorant binding proteins. A nanosensor combining localized SPR and small odorant binding proteins from honeybees has been established in which the detection range is 10 nM - 1 mM using a quantitative array of nanocups. To monitor and preserve a stable environment for marine food systems, marine contaminant detection is used. Finally, through their sensitive detection capabilities, miniaturized devices, wireless communication, and small-scale networks, biosensors can be applied to marine food safety to be established as advanced analytical and monitoring tools.

Another development kit for food safety biosensors focuses on the detection of genetically modified organisms (GMOs) in food products. Since the 1990s, GMOs in all fields of agricultural products have been considered a biotechnology revolution. To present, more than 45 percent of the world's soybeans, 40 percent of corn, and 50 percent of cotton are GM products; and GM is also used in livestock. Recent research, however, indicates that GMO products can affect human and animal bodies through gastrointestinal problems, antibiotic resistance, allergenicity, diversity of farm products degradation, and undesired gene flow to other species. Biosensors are designed to measure GMOs in foods and feeds using isothermal DNA amplification and fast detection signal detection to identify GM genes. Detecting unidentified DNA genes that can be resolved by high-throughput technology like the combination of biosensing and arrays, and the development of databases of GMO genes are the key challenges in GMO detection.

BIOSENSORS & BIOCHIPS FOR ENVIRONMENTAL MONITORING

Due to the strong connection between environmental pollution and human health/ socioeconomic progress, environmental monitoring has become one of the priorities on a European and global scale. Biosensors have been commonly used as cost-effective, rapid, *in situ*, and real-time analytical techniques in this field. The recent development of biosensors with new transduction materials obtained from nanotechnology and for multiplexed pollutant detection, involving multidisciplinary experts, explains the need for compact, fast, and smart biosensing devices. Several recent developments exist in the monitoring of air, water, and soil contaminants by biosensors under real conditions, like pesticides,



highly toxic components and small organic molecules, including toxins and endocrine disrupting chemicals.

Biosensors used in environmental monitoring can be categorized as optical (including optical fibre and surface plasmon resonance biosensors), electrochemical (including amperometric and impedance biosensors) and piezoelectric (including quartz crystal microbalance biosensors) based on their transduction or as immunosensors, aptasensors, genosensors and enzymatic biosensors based on their recognition elements, respectively when are used antibodies, aptamers, nucleic acids, and enzymes. The majority of biosensors in environmental monitoring are recognized as immunosensors and enzymatic biosensors, but the development of aptasensors has recently increased due to the beneficial characteristics of aptamers, like ease of modification, thermal stability, *in vitro* synthesis and the ability to design their structure, to differentiate targets with different functional groups and to rehybridize.

Study on the design of biosensors for the monitoring of organic pollutants, potentially toxic elements and pathogens in the environment has led to the sustainable development of civilization due to the environmental pollution issues confronting human health. Various chromatographic techniques (such as gas chromatography and high-performance liquid chromatography combined with capillary electrophoresis or mass spectrometry) are conventional analytical methods used for environmental monitoring of pollutants, but they require costly reagents, time-consuming sample pre-treatment and costly equipment. Therefore, for monitoring pollutants responsible for adverse effects on habitats and human health, more sensitive, cost-effective, fast, easy to function, and compact biosensing devices are desperately needed to overcome the magnification of environmental issues. In the case of accidental release of pesticides or acute poisoning, for example, common methods are not appropriate for *in situ* measurements where fast, miniaturized, and portable equipment like environmental monitoring biosensors is required. In this regard, the role of nanotechnology in the creation of rapid and intelligent biosensing devices is crucial for the success of environmental pollutant detection; most recent biosensors include nanomaterials and novel nanocomposites in their systems, which are beneficial for improving analytical performance, such as sensitivity and detection limits.

For the detection and monitoring of different environmental pollutants, biosensors, including immunosensors, aptasensors, genosensors and enzymatic biosensors have been documented using antibodies, aptamers, nucleic acids, and enzymes as recognition elements.

1. Pesticides

Pesticides are among the most significant environmental pollutants because of their large presence in the environment. Organophosphorus insecticides, for example, are commonly used in agriculture and represent a group of pesticides which, due to their high toxicity, are of immense environmental concern. Easy, responsive, and miniaturized *in situ* methodologies like biosensors have



2019-1-BG01-KA203-062371

therefore been established as analytical strategies for their detection and monitoring, without the need for comprehensive sample pre-treatment.

Disposable amperometric enzymatic (acetylcholinesterase) biosensors were proposed for the detection of organophosphorus insecticides using paraoxon as a model analyte applying a cysteamine self-assembled monolayer on gold screen-printed electrodes. The disposable biosensors showed a linear spectrum of up to 40 ppb with a 2 ppb detection limit and a $113 \mu\text{A mM cm}^{-2}$ sensitivity. Using the self-assembled monolayer, good analytical output could be due to the highly oriented enzyme immobilization. Recoveries of 97 ± 5 percent ($n = 3$) were reported after being tested in river water samples spiked with 10 ppb of paraoxon, indicating the effectiveness of such enzymatic biosensors. Furthermore, the use of disposable screen-printed electrodes dispenses with time-consuming methods like the reactivation of immobilized enzymes utilizing, for example, obidoxime solution and pralidoxime iodide (PAM) or the use of the renewable enzyme membrane needed for the second application of biosensors.

Nanoparticles based on iridium oxide have been used in the disposable enzymatic biosensor with tyrosinase based on low-cost screen-printed carbon electrodes for the detection of chlorpyrifos in river water samples. Linear biosensor response ($0.01\text{--}0.1 \mu\text{M}$) and low detection limit (3 nM) were reported, which could be due to the high conductivity of nanoparticles of iridium oxide and tyrosinase efficiency. Recovery tests were carried out in river water samples with the addition of $0.1 \mu\text{M}$ of chlorpyrifos and recoveries of 90 ± 9.6 percent were obtained with a residual standard deviation (RSD) smaller than 10 percent ($n = 3$) to demonstrate the applicability of the biosensor.

Acetamiprid was detected by colorimetric aptasensors and water samples by impedimetric aptasensors in real environmental samples, like fresh surface soil samples. A linear range of 75 nM to $7.5 \mu\text{M}$ and a detection limit of 5 nM were observed with the colorimetric aptasensor, while a wider linear range (50 fM to $10 \mu\text{M}$) and a lower detection limit (17 fM) were observed with the impedimetric aptasensor. Gold nanoparticles, multi-walled carbon nanotubes (MWCNT) and reduced graphene oxide nanoribbons were used in that biosensor as a composite to sustain the electrode surface acetamiprid aptamer, which could be responsible for higher electron transfer and improved analytical performance of the biosensor. A related detection limit (33 fM) was observed by an aptasensor based on silver nanoparticles anchored on nitrogen-doped nanocomposite graphene oxide constructed for acetamiprid detection in wastewater samples.

2. Pathogens

The existence of pathogens in environmental matrices, and especially in water compartments, could pose a serious risk to human health, and some biosensors have recently been suggested for monitoring the environment. For example, for the detection of metabolically active *Legionella pneumophila* in complex environmental water samples, rapid and precise optical biosensors based on surface plasmon resonance have been proposed. In one study, the detection principle was based on the

identification of bacterial RNA by the immobilized RNA detector probe on the gold surface of the biochip. For signal amplification, streptavidin-conjugated quantum dots were used, and the detection period was approximately three hours, indicating the viability of the biosensing device for successful bacteria detection in the range of 10^4 - 10^8 CFU mL⁻¹.

3. Potentially toxic elements

The contamination by heavy metals and corresponding ions of the natural waters can pose significant risks to human health, and compact, low-cost, and rapid heavy metal analyses are a global priority concern. As a model target for testing an optical DNA biosensor for the detection of heavy metal ions that are extremely toxic and common pollutants in the environment, mercury ions (Hg^{2+}) were used. The biosensor was compact, low-cost, and rapid with *in situ* screening of Hg^{2+} in natural waters in less than 10 min. The detection principle is focused on the capacity of certain metal ions to bind selectively to certain bases to form stable metal-mediated DNA duplexes; in the case of Hg^{2+} , thymine bases can be selectively coordinated to form stable thymine- Hg^{2+} -thymine complexes. In the detection range between 0 and 1000 nM, a detection limit of 1.2 nM was achieved, which is lower than the maximum value requested by the United States Environmental Protection Agency (10 nM)

For the detection of Pb^{2+} in water samples (pond and lake water samples) using DNAzymes/carboxylated magnetic beads and DNA aptamers, two fluorescence based optical biosensors have recently been suggested. The detection limits of 5 nM and 61 nM, respectively, were observed by biosensors based on DNAzymes and DNA aptamers, with a respective linear detection range of 0 to 50 nM and 100 to 1000 nM. The use of label-free unique dye (SYBER Green I), which was intercalated with double stranded DNA, showing strong fluorescence intensities, as seen in Figure 5. Moreover, the absence of biosensor fluorescence intensity is observed only with the dye (curve a). With the DNAzyme + Pb^{2+} (curve b) the fluorescence intensity increases with the addition of the dye + DNAzyme + Pb^{2+} , illustrating the sensitivity of the biosensor towards Pb^{2+} .

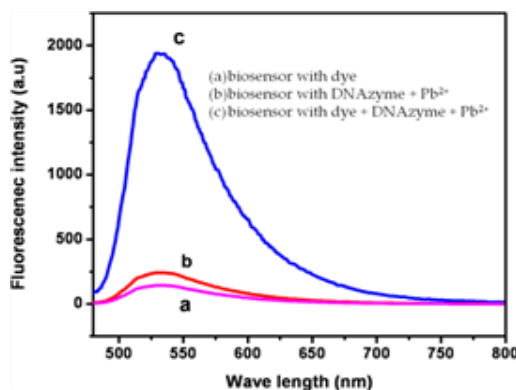


Figure 5. Fluorescence emission spectra for detection of Pb^{2+}



2019-1-BG01-KA203-062371

4. Toxins

Harmful toxins like brevetoxins and microcystins are created by the eutrophication of aquatic systems by the algal blooms of cyanobacteria, and thus accurate and cost-effective systems are needed for the early detection of such toxins. For the sensitive detection of brevetoxin-2, a marine neurotoxin, an electrochemical aptasensor has been used composed by gold electrodes functionalized with cysteamine self-assembled monolayers. A detection limit of 106 pg mL^{-1} was achieved and strong selectivity was observed for brevetoxin-2 against other toxins of various groups, like okadaic acid and microcystin. The feasibility of the aptasensor for detecting brevetoxin-2 in real samples was achieved by analysing shellfish and strong recoveries (102-110 percent) were reported, indicating no interaction with the aptasensor response from the shellfish matrix.

5. Endocrine disrupting chemicals

In water samples, bisphenol A was detected as an endocrine disrupting chemical by aptasensors based on the fluorescence principle with functionalized aptamers (fluorescein amidite) and gold nanoparticles and based on evanescent-wave optical fibre. The evanescent-wave optical fibre aptasensor was compact and found to be rapid, cost-effective, sensitive and selective for the detection of bisphenol A in water samples, with the benefit of no requirement of any pre-concentration or treatment steps. Furthermore, the aptasensor can be reused for 90 s by regeneration with a 0.5% sodium dodecyl sulphate (SDS) solution and further washing with a phosphate buffered saline (PBS) solution (pH 7.2) for over a hundred assay cycles without any noticeable loss of efficiency. Similar detection limits (0.1 and 0.45 ng mL^{-1}) were observed in both optical biosensors where the DNA molecule probe, which is the complementary sequence of a small fraction of the bisphenol A aptamer, was adsorbed by electrostatic interaction in the surface of gold nanoparticles and covalently immobilized on the surface of the fibre. Lately, for the detection of bisphenol A in river water samples using molybdenum carbide nanotubes, another fluorescence-based aptasensor was proposed. With such a label-free, inexpensive, and easy to use aptasensor, a low detection limit of 0.23 ng mL^{-1} has been obtained. The specificity of the aptasensor was evaluated by analysing other molecules with structures similar to that of bisphenol A (e.g., 4,4J-biphenol, bisphenol AF, and 4,4J-sulfonyldiphenol) and only background signals showing high specificity for bisphenol A were identified for these molecules.

A disposable and label-free electrochemical immunosensor based on a field effect transistor with SWCNT has recently been employed in seawater samples for assessing another endocrine disrupting chemical - 4-nonylphenol. The immunosensor has a high reproducibility ($0.56 \pm 0.08\%$), an average recovery of 97.8% to 104.6% and a low detection limit ($5 \text{ } \mu\text{g L}^{-1}$), which is lower than the recommended maximum concentration of $7 \text{ } \mu\text{g L}^{-1}$ specified by the corresponding regulations. In seawater samples such as 4-nonylphenol, the biosensor could be used to detect hazardous priority substances, even at low concentrations and with an easy and low-cost methodology.



6. Other environmental compounds

New, fast, and accurate analytical methodologies have been needed for the early detection and monitoring of various other hazardous compounds liberated during algal blooms. Due to the excellent sensitivity and specificity of nucleic acid probes to their complementary binding partners, biosensors have been developed to detect algal RNA. For the enhanced selective and sensitive detection of RNA from 13 harmful algal organisms, an electrochemical genosensor based on screen-printed gold electrode was recently reported; the genosensor could distinguish RNA targets from environmental samples (spiked seawater samples) containing 10^5 cells, considered to be the limit of detection.

References

- Adami A; Mortari A; Morganti E; Lorenzelli L. 2018. Microfluidic Sample Preparation Methods for the Analysis of Milk Contaminants. Available online: <https://www.hindawi.com/journals/js/2016/2385267/>
- Al-Mawali A. 2015. Non-communicable diseases: shining a light on cardiovascular disease, Oman's biggest killer. *Oman Med. J.*, 30 (4): 227.
- Arduini F, Guidone S, Amine A, Palleschi G, Moscone D. 2013. Acetylcholinesterase biosensor based on self-assembled monolayer-modified gold-screen printed electrodes for organophosphorus insecticide detection. *Sens. Actuators B Chem.*, 179: 201–208.
- Arduini F, Cinti S, Scognamiglio V, Moscone D. 2016. Nanomaterials in electrochemical biosensors for pesticide detection: advances and challenges in food analysis. *Microchim. Acta*, 183: 2063–2083.
- Arugula MA, Simonian AL. 2016. Biosensors for Detection of Genetically Modified Organisms in Food and Feed. In *Genetically Modified Organisms in Food*, Elsevier: Amsterdam, The Netherlands, pp. 97–110, ISBN 978-0-12-802259-7.



2019-1-BG01-KA203-062371

- Ayari-Jeridi H. et al. 2015. Mutation spectrum of RB1 gene in unilateral retinoblastoma cases from Tunisia and correlations with clinical features. *PLoS One*, 10 (1): e0116615.
- Bahadır EB, Sezgentürk MK. 2017. Biosensor technologies for analyses of food contaminants. In *Nanobiosensors*, Elsevier: Amsterdam, The Netherlands, pp. 289–337, ISBN 978-0-12-804301-1.
- Baldwin CJ. 2015. Introduction to the Principles. In *The 10 Principles of Food Industry Sustainability*, John Wiley & Sons, Ltd.: Hoboken, NJ, USA, pp. 1–14, ISBN 978-1-118-44769-7.
- Beck MB, Walker VR. 2013. On water security, sustainability, and the water-food-energy-climate nexus. *Front. Environ. Sci. Eng.*, 7: 626–639.
- Belkhamssa N, da Costa JP, Justino CIL, Santos PSM, Cardoso S, Duarte AC, Rocha-Santos T, Ksibi M. 2016. Development of an electrochemical biosensor for alkylphenol detection. *Talanta*, 158: 30–34.
- Bhalla N. et al. 2016. Introduction to biosensors. *Essays Biochem.*, 60 (1): 1–8.
- Bohunicky B, Mousa SA. 2011. Biosensors: the new wave in cancer diagnosis. *Nanotechnol. Sci. Appl.*, 4: 1.
- Bourne MC. 2014. Food Security: Postharvest Losses. In *Encyclopedia of Agriculture and Food Systems*, Elsevier: Amsterdam, The Netherlands, pp. 338–351, ISBN 978-0-08-093139-5.
- Bruen D et al. 2017. Glucose sensing for diabetes monitoring: recent developments. *Sensors*, 1866.
- Burris KP, Stewart CN. 2012. Fluorescent nanoparticles: Sensing pathogens and toxins in foods and crops. *Trends Food Sci. Technol.*, 28: 143–152.
- Byrne B et al. 2009. Antibody-based sensors: principles, problems and potential for detection of pathogens and associated toxins. *Sensors*, 9 (6): 4407–4445.
- Cash KJ, Clark HA. 2010. Nanosensors and nanomaterials for monitoring glucose in diabetes. *Trends Mol. Med.*, 16 (12): 584–593.
- Chao R, Mishra S, Si T, Zhao H. 2017. Engineering biological systems using automated biofoundries. *Metab. Eng.*, 42: 98–108.
- Chen Y, Li H, Gao T, Zhang T, Xu L, Wang B, Wang J, Pei R. 2018. Selection of DNA aptamers for the development of light-up biosensor to detect Pb(II). *Sens. Actuators B Chem.*, 254: 214–221.
- Eissa S, Siaj M, Zourob M. 2015. Aptamer-based competitive electrochemical biosensor for brevetoxin-2. *Biosens. Bioelectron.*, 69: 148–154.
- Elmore S. 2007. Apoptosis: a review of programmed cell death. *Toxicol. Pathol.*, 35 (4): 495–516.



2019-1-BG01-KA203-062371

Enrico DL, Manera MG, Montagna G, Cimaglia F, Chesa M, Poltronieri P, Santino A, Rella R. 2013. PR based immunosensor for detection of *Legionella pneumophila* in water samples. *Opt. Commun.*, 294: 420–426.

EPA. National Recommended Water Quality Criteria-Aquatic Life Criteria Table. Available online: <http://www.epa.gov/wqc/national-recommended-water-quality-criteria-aquatic-life-criteria-table>

Fei A, Liu Q, Huan J, Qian J, Dong X, Qiu B, Mao H, Wang K. 2015. Label-free impedimetric aptasensor for detection of femtomole level acetamiprid using gold nanoparticles decorated multiwalled carbon nanotube-reduced graphene oxide nanoribbon composites. *Biosens. Bioelectron.*, 70: 122–129.

Foudeh AM, Trigui H, Mendis N, Faucher SP, Veres T, Tabrizian M. 2015. Rapid and specific SPRi detection of *L. pneumophila* in complex environmental water samples. *Anal. Bioanal. Chem.*, 407: 5541–5545.

Garnet TF. 2013. Food sustainability: Problems, perspectives and solutions. *Proc. Nutr. Soc.*, 72: 29–39.

Gheorghe I, Czobor I, Lazar V, Chifiriuc MC 2017. Present and perspectives in pesticides biosensors development and contribution of nanotechnology. In *New Pesticides and Soil Sensors*, Elsevier: Amsterdam, The Netherlands, pp. 337–372, ISBN 978-0-12-804299-1.

Ghorashi M. 2018. Technology's Role in Eradicating Foodborne Illness. Available online: <https://www.foodsafetymagazine.com/signature-series/technologye28099s-role-in-eradicating-foodborne-illness/>

Giacinti C, Giordano A. 2006. RB and cell cycle progression. *Oncogene*, 25 (38): 5220-5227.

Guo L, Li Z, Chen H, et al. 2017. Colorimetric biosensor for the assay of paraoxon in environmental water samples based on the iodine-starch color reaction. *Anal. Chim. Acta*, 967: 59–63.

Hameed I, et al. 2015. Type 2 diabetes mellitus: from a metabolic disorder to an inflammatory condition. *World J. Diabetes*, 6 (4): 598.

Hassani S, Momtaz S, Vakhshiteh F, et al. 2017. Biosensors and their applications in detection of organophosphorus pesticides in the environment. *Arch. Toxicol.*, 91: 109–130.

He MQ, Wang K, Wang J, Yu YL, He RH. 2017. A sensitive aptasensor based on molybdenum carbide nanotubes and label-free aptamer for detection of bisphenol A. *Anal. Bioanal. Chem.*, 409: 1797–1803.

Holford TR et al. 2012. Recent trends in antibody based sensors. *Biosens. Bioelectron.*, 34 (1): 12–24.



2019-1-BG01-KA203-062371

- Hughes RA, Ellington AD. 2017. Synthetic DNA synthesis and assembly: Putting the synthetic in synthetic biology. *Cold Spring Harb. Perspect. Biol.*, 9.
- Husu I, Rodio G, Touloupakis E, et al. 2013. Insights into photo-electrochemical sensing of herbicides driven by *Chlamydomonas reinhardtii* cells. *Sens. Actuators B Chem.*, 185: 321–330.
- Jain KK. 2004. “Applications of biochips: from diagnostics to personalized medicine.” *Curr Opin Drug Discov Devel*, 7(3): 285-289.
- Jiang D, Du X, Liu Q, Zhou L, Dai L, Qian J, Wang K. 2015. Silver nanoparticles anchored on nitrogen-doped graphene as a novel electrochemical biosensing platform with enhanced sensitivity for aptamer-based pesticide assay. *Analyst*, 140: 6404–6411.
- Justino CIL, Freitas AC, Duarte AC, Santos TAPR. 2015. Sensors and biosensors for monitoring marine contaminants. *Trends Environ. Anal. Chem.*, 6–7: 21–30.
- Justino CIL, Freitas AC, Pereira R, Duarte AC, Rocha-Santos TAP. 2015. Recent developments in recognition elements for chemical sensors and biosensors. *Trends Anal. Chem.*, 68: 2–17.
- Kazemi-Darsanaki R et al. 2012. Biosensors: functions and applications. *J. Biol. Today's World*, 2 (1): 20–23.
- Khot LR, Sankaran S, Maja JM, Ehsani R, Schuster EW. 2012. Applications of nanomaterials in agricultural production and crop protection: A review. *Crop Prot.*, 35: 64–70.
- Kost GJ, Tran NK., 2005. Point-of-care testing and cardiac biomarkers: the standard of care and vision for chest pain centers. *Cardiol. Clin.*, 23 (4): 467–490.
- Lang Q, Han L, Hou C, Wang F, Liu A. 2016. A sensitive acetylcholinesterase biosensor based on gold nanorods modified electrode for detection of organophosphate pesticide. *Talanta*, 156: 34–41.
- Lee EY, Muller WJ. 2010. Oncogenes and tumor suppressor genes. *Cold Spring Harb. Perspect. Biol.*, 2 (10): a003236.
- Li Z, Yu Y, Li Z, Wu T. 2015. A review of biosensing techniques for detection of trace carcinogen contamination in food products. *Anal. Bioanal. Chem.*, 407: 2711–2726.
- Liao W, Lu X. 2016. Determination of chemical hazards in foods using surface-enhanced Raman spectroscopy coupled with advanced separation techniques. *Trends Food Sci. Technol.*, 54: 103–113.
- Long F, Zhu A, Shi H, Wang H, Liu J. 2013. Rapid on-site/in-situ detection of heavy metal ions in environmental water using a structure-switching DNA optical biosensor. *Sci. Rep.*, 3: 2308.
- Loo C, et al. 2005. Immunotargeted nanoshells for integrated cancer imaging and therapy. *Nano Lett.*, 5 (4): 709–711.



2019-1-BG01-KA203-062371

Maduraiveeran G, Jin W. 2017. Nanomaterials based electrochemical sensor and biosensor platforms for environmental applications. *Trends Environ. Anal. Chem.*, 13: 10–23.

Marcellin E, Nielsen LK 2018. Advances in analytical tools for high throughput strain engineering. *Curr. Opin. Biotechnol.*, 54: 33–40.

Martín-Timón I et al. 2014. Type 2 diabetes and cardiovascular disease: have all risk factors the same strength? *World J. Diabetes*, 5 (4): 444.

Mayorga-Martinez C, Pino F, Kurbanoglu S, et al. 2014. Iridium oxide nanoparticles induced dual catalytic/inhibition based detection of phenol and pesticide compounds. *J. Mater. Chem. B*, 2: 2233–2239.

McPartlin DA, Loftus JH, Crawley AS, et al. 2017. Biosensors for the monitoring of harmful algal blooms. *Curr. Opin. Biotechnol.*, 45: 164–169.

Mehrotra P. 2016. Biosensors and their applications - a review. *J. Oral. Biol. Craniofac Res.*, 6 (2): 153–159.

Meriç S, Çakır Ö, Turgut-Kara N, Arı S. 2014. Detection of genetically modified maize and soybean in feed samples. *Genet. Mol. Res.*, 13: 1160–1168.

Moran KLM, Fitzgerald J, McPartlin DA, Loftus JH, O’Kennedy R. 2016. Biosensor-Based Technologies for the Detection of Pathogens and Toxins. In *Comprehensive Analytical Chemistry*, Elsevier: Amsterdam, The Netherlands, Volume 74, pp. 93–120, ISBN 978-0-444-63579-2.

Mungroo NA, Neethirajan S. 2014. Biosensors for the Detection of Antibiotics in Poultry Industry—A Review. *Biosensors*, 4: 472–493.

Ngoepe M et al. 2013. Integration of biosensors and drug delivery technologies for early detection and chronic management of illness. *Sensors*, 13 (6): 7680–7713.

Omidfar K. et al. 2013. New analytical applications of gold nanoparticles as label in antibody based sensors. *Biosens. Bioelectron.*, 43: 336–347.

Orozco J, Villa E, Manes C, Medlin LK, Guillebault D. 2016. Electrochemical RNA genosensors for toxic algal species: Enhancing selectivity and sensitivity. *Talanta*, 161: 560–566.

Patra S, Roy E, Madhuri R, Sharma PK. 2017. A technique comes to life for security of life: The food contaminant sensors. In *Nanobiosensors*, Elsevier: Amsterdam, The Netherlands, pp. 713–772, ISBN 978-0-12-804301-1.



2019-1-BG01-KA203-062371

Pola-López LA, Camas-Anzueto JL, Martínez-Antonio A, et al. 2018. Novel arsenic biosensor “POLA” obtained by a genetically modified *E. coli* bioreporter cell. *Sens. Actuators B Chem.*, 254: 1061–1068.

Ragavan KV, Selvakumar LS, Thakur MS. 2013. Functionalized aptamers as nano-bioprobes for ultrasensitive detection of bisphenol-A. *Chem. Commun.*, 49: 5960–5962.

Rapini R, Marrazza G. 2016. Biosensor Potential in Pesticide Monitoring. In *Comprehensive Analytical Chemistry*, Elsevier: Amsterdam, The Netherlands, Volume 74, pp. 3–31, ISBN 978-0-444-63579-2.

Ravikumar A, Panneerselvam P, Radhakrishnan K, et al. 2017. DNzyme based amplified biosensor on ultrasensitive fluorescence detection of Pb(II) ions from aqueous system. *J. Fluoresc.*, 27: 2101–2109.

Rocchitta G, et al. 2016. Enzyme biosensors for biomedical applications: strategies for safeguarding analytical performances in biological fluids. *Sensors*, 16 (6).

Rotariu L, Lagarde F, Jaffrezic-Renault N, Bala C. 2016. Electrochemical biosensors for fast detection of food contaminants—Trends and perspective. *TrAC Trends Anal. Chem.*, 79: 80–87.

Saucedo NM, Mulchandani A. 2016. Sensing of Biological Contaminants. In *Comprehensive Analytical Chemistry*, Elsevier: Amsterdam, The Netherlands, Volume 74, pp. 73–91, ISBN 978-0-444-63579-2.

Shi H, Zhao G, Liu M, Fan L, Cao T. 2013. Aptamer-based colorimetric sensing of acetamiprid in soil samples: Sensitivity, selectivity and mechanism. *J. Hazard. Mater.*, 260: 754–761.

Singh M, del Valle M. 2015. Arsenic Biosensors. In *Handbook of Arsenic Toxicology*, Elsevier: Amsterdam, The Netherlands, pp. 575–588, ISBN 978-0-12-418688-0.

Sinha K, Ghosh J, Sil PC. 2017. New pesticides: A cutting-edge view of contributions from nanotechnology for the development of sustainable agricultural pest control. In *New Pesticides and Soil Sensors*, Elsevier: Amsterdam, The Netherlands, pp. 47–79, ISBN 978-0-12-804299-1.

Sodano V, Gorgitano MT, Quaglietta M, Verneau F. 2016. Regulating food nanotechnologies in the European Union: Open issues and political challenges. *Trends Food Sci. Technol.*, 54: 216–226.

Specht K, Siebert R, Hartmann I, et al. 2014. Urban agriculture of the future: An overview of sustainability aspects of food production in and on buildings. *Agric. Hum. Values*, 31: 33–51.

Stadler RH. 2016. Foreword for Food Processing—Derived Contaminants in Food Analysis. In *Reference Module in Food Science*, Elsevier: Amsterdam, The Netherlands, ISBN 978-0-08-100596-5.



2019-1-BG01-KA203-062371

- Tabish SA. 2007. Is diabetes becoming the biggest epidemic of the twenty-first century? *Int J. Health Sci.* 1 (2), V–VIII.
- Templier V, Roux A, Roupioz Y, Livache T. 2016. Ligands for label-free detection of whole bacteria on biosensors: A review. *TrAC Trends Anal. Chem.*, 79: 71–79.
- Thomas S, et al. 2015. The expression of retinoblastoma tumor suppressor protein in oral cancers and precancers: a clinicopathological study. *Dent. Res. J.*, 12 (4): 307.
- Tian L, Hires SA, Mao T, et al. 2009. Imaging neural activity in worms, flies and mice with improved GAAmp calcium indicators. *Nat. Methods*, 6: 875–881.
- Turner AP. 2013. Biosensors: Sense and sensibility. *Chem. Soc. Rev.*, 42: 3184–3196.
- USEPA. Mercury Update: Impact of Fish Advisories, EPA Fact Sheet EPA-823-F-01-011, EPA, Office of Water: Washington, DC, USA, 2001.
- Valastyan S, Weinberg RA. 2011. Tumor metastasis: molecular insights and evolving paradigms. *Cell*, 147 (2): 275–292.
- Verma N, Kaur G. 2016. Trends on Biosensing Systems for Heavy Metal Detection. In *Comprehensive Analytical Chemistry*, Elsevier: Amsterdam, The Netherlands, Volume 74, pp. 33–71, ISBN 978-0-444-63579-2.
- Vu T, Claret FX. 2012. Trastuzumab: updated mechanisms of action and resistance in breast cancer. *Front. Oncol.*, 2: 62.
- Way JC, Collins JJ, Keasling JD, Silver PA. 2014. Integrating biological redesign: Where synthetic biology came from and where it needs to go. *Cell*, 157: 151–161.
- Weng X, Neethirajan S. 2017. Ensuring food safety: Quality monitoring using microfluidics. *Trends Food Sci. Technol.*, 65: 10–22.
- Xiao Y, Lubin AA, Heeger AJ, Plaxco KW. 2005. Label-free electronic detection of thrombin in blood serum by using an aptamer-based sensor. *Angew. Chem. Int. Ed. Engl.*, 44: 5456–5459.
- Yildirim N, Long F, He M, Shi HC, Gu AZ. 2014. A portable optic fiber aptasensor for sensitive, specific and rapid detection of bisphenol-A in water samples. *Environ. Sci. Process Impacts*, 16: 1379–1386.
- Yoshida K, Miki Y. 2004. Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage. *Cancer Sci.*, 95 (11): 866–871.
- Zhang D, Lu Y, Zhang Q, et al. 2015. Nanoplasmonic monitoring of odorants binding to olfactory proteins from honeybee as biosensor for chemical detection. *Sens. Actuators B Chem.*, 221: 341–349.



2019-1-BG01-KA203-062371

Zhang F, Zhang Q, Zhang D, Lu Y, Liu Q, Wang P. 2014. Biosensor analysis of natural and artificial sweeteners in intact taste epithelium. *Biosens. Bioelectron.*, 54: 385–392.

Zhang W, Asiri AM, Liu D, Du D, Lin Y. 2015. Nanomaterial-based biosensors for environmental and biological monitoring of organophosphorus pesticides and nerve agents. *Trends Anal. Chem.*, 54: 1–10.



Project website: www.digit-biotech.eu

The European Commission's support for the production of this publication does not constitute an endorsement of the contents, which reflect the views only of the authors, and the Commission cannot be held responsible for any use which may be made of the information contained therein.



**LO6 SOCIAL AND
POLITICAL ACCEPTABILITY
OF MODERN
BIOTECHNOLOGY TOOLS**

Basic level

AUTHOR:

TRAYANA NEDEVA & ANNA KUJUMDZIEVA



Contents

Modern Biotechnology at a Glance.....	3
Practical Applications of Modern Biotechnology	4
Social Attitude to Modern Biotechnology	5
Societal Impact.....	6
Establishment and spreading of knowledge	7
Science and Technology (S&T) impact on society	7
Perception of modern biotechnology applications	8
Benefits and Risks from Biotechnology Applications	12
Benefits vs. risks societal dispute.....	12
Risk acceptance.....	13
Risk Management.....	15
Social benefits of modern biotechnology.....	15
Acceptance and diffusion of modern biotechnology.....	16
Possible negative impacts of modern biotechnology	19
Socioeconomical Impacts.....	19
Impacts on biodiversity and sustainable agriculture	19
Impacts on public welfare	20
Biosafety issues of modern biotechnology.....	20
Conclusions	21
References	22



Modern Biotechnology at a Glance

Biotechnology is a modern advanced technology grounded on science disciplines like molecular biology, biochemistry, cell biology. It accepts a lot of new techniques such as cell and tissue cultivation, cell fusion, gene recombination, microbial fermentation, and also duplication and recombination of organisms at a cellular, chromosomal, and gene level. In this way, it can make organisms answering to human needs, produce new products and breed new plants having novel features of high output and stress resistance. Modern biotechnology is composed of four technological systems. They are genetic, cell, enzyme, and fermentation engineering, of which genetic engineering and cell engineering are the most advanced ones.

Besides the great social and economic benefits of modern biotechnology, there is a possibility it to harm human health and the environment. This can result in many socio-economic problems, such as destroying original social and economic patterns, posing threats to biodiversity and traditional crop varieties, injuring countries or communities' socioeconomic welfare, violating traditional ethical, moral, and religious values.

The modern biotechnological practice in the production of fiber, pharmaceuticals, and food has a powerful development of the late 20th and beginning of the 21st century. This emerging technology is often accepted as the next technological revolution possessing the potential to change fundamentally the way of societal arrangement regarding production and distribution of goods. Considerable investments have been already made in biotechnological research for new products' development. Currently, science and technology are expected to bring consumers a big variety of genetically modified (GM) products. Besides, many GM products have already entered the food distribution chains. Despite its promise to bring significant benefits to society, public acceptance of modern biotechnology is quite diverse at the world dimension. To enable biotechnology to contribute major benefits for human beings, the international society has paid serious attention to the biosafety of the overall biotechnological production to promote its better acceptance.



Practical Applications of Modern Biotechnology

Nowadays, biotechnology has been classified into few categories based on its applications. They are depicted in Fig. 1.

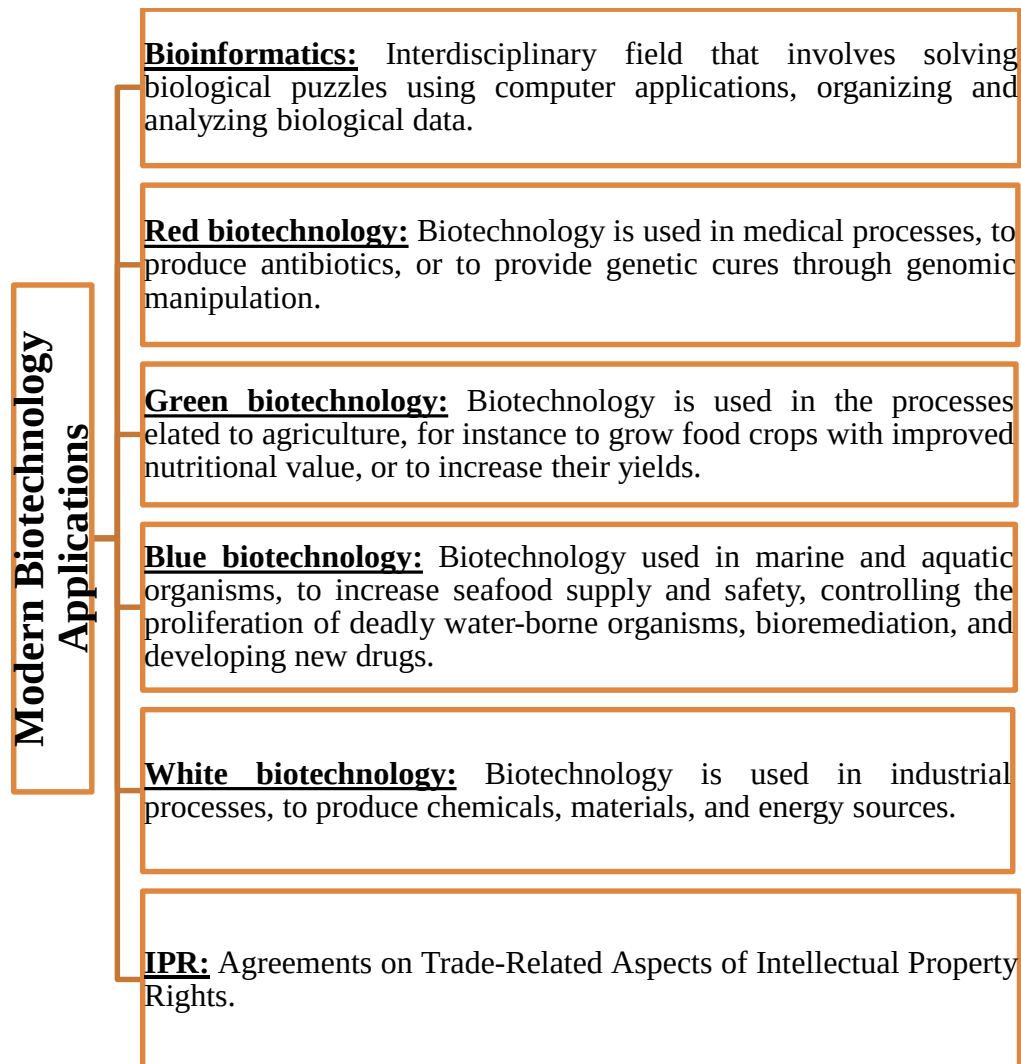


Figure 1. Practical applications of modern biotechnology



Social Attitude to Modern Biotechnology

In general, biotechnology intends to emphasize the potential benefits to society through reduction of hunger and malnutrition, suspension and healing of diseases, and increase of health and general well-being. On the other hand, there is an opinion that GM products are used as a needless interference with nature and can cause unknown and potentially disastrous consequences. At the same time in the U.S., GM crops entered the grain supply chains without raising major public concern. Besides, agricultural biotechnology is facing significant opposition in Europe and many other developed countries. Regarding the obvious public troubles about the perceived risks for humans and the environment, European Union (EU) accepted rather limited regulations on all transgenic crops in any part of the EU food system. At a global level, the situation is even more diverse: in the U.K., objection movements throw down GM crops on different occasions. Until recently, Brazil and India refused to approve any GM crop. Due to similar consumer concerns, some fast-food supply chains decided not to use GM potato in their products.

The reason against the use of gene technologies in agricultural production is grounded on the fact that some people and institutions see risks to humans and the environment, while others oppose it minding moral, ethical, and social concerns. Biotechnology frequently is running down because of its tools' application in plants and animals, especially gene transfer across species, based on understanding "realms of God" and against "Law of Nature". Also, there is an opinion that since genes are naturally occurring parts, which are an object of discovery (not of invention), patent ownership of genetic findings and processes is morally and ethically insolvent.

Public discussions on agricultural biotechnology also pose some social and political debates. There is a talk concerning the application of modern genetic technologies in production of commodities in developed countries that are currently imported to developing countries. It is claimed that such developments will have significant negative effects on the poverty situation in the Third world and will lead to global instability. At the same time, other researchers have the opposite opinion. Another source of care is the possibility that farmers will become steadily dependent on multinational corporations for their "means of production" bringing harmful economic, social and political effects.

The importance of this subject defines the need for a full understanding of public interests and concerns regarding an agreement between private and public decisions concerning food biotechnology. Consumer acceptance of biotechnology is strongly related not only to expected risks and benefits associated with GM products but also to their moral and ethical dimension. Further, public views about multinational corporations, trust in government, science, and technology development also reveal their attitudes towards biotechnology. It is found that consumers' cognitive factors (e.g., levels of risk aversion, opinions about GM foods) impacted the acceptance of GM food products, while the socio-economic factors did not have significant effects. Thus, the public perceptions of biotechnology possess a lot of dimensions and are possibly affected by multiple forces, preferences, and events. For instance, positive benefits (e.g., nutritional benefits from improved/new products, environmental benefits via reduced use of pesticides, etc.) can encourage consumer acceptance of food biotechnology. On the other hand, sensibility about risks to humans and the environment is expected to have adverse effects on public

acceptance of GM products. Thus, the activities to analyze and determine the factors influencing consumer attitudes towards biotechnology have to follow the procedure, denoted in Fig. 2.

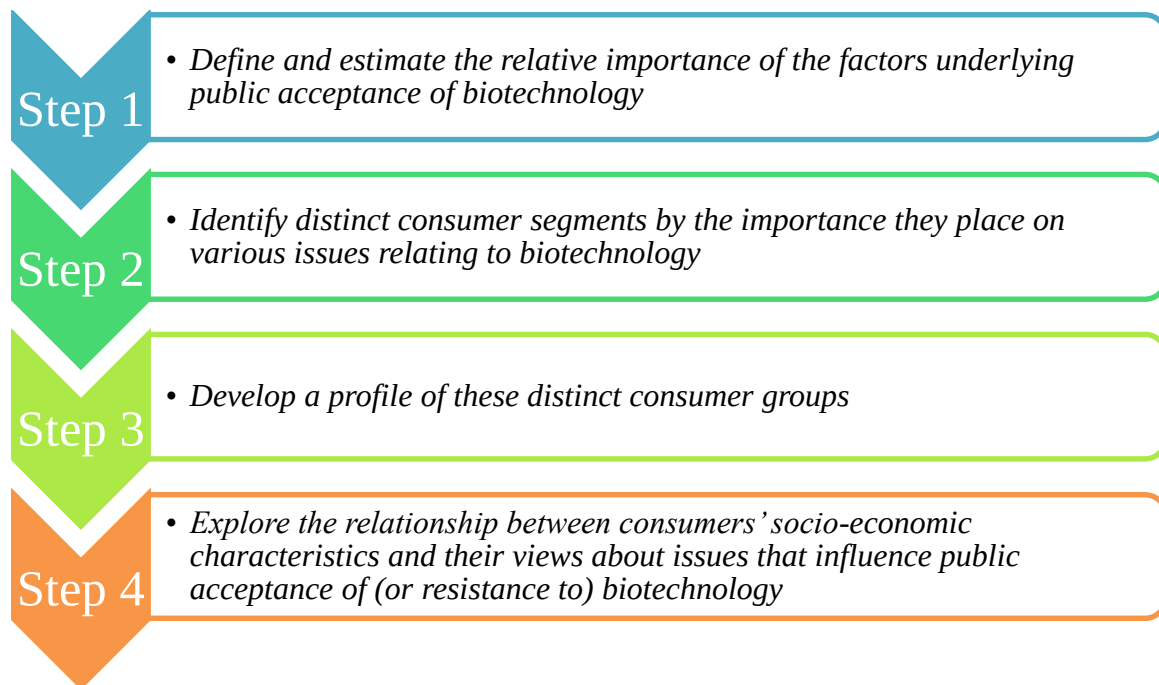


Figure 2. The procedure for analysis and determination of the factors influencing consumer attitudes towards biotechnology

SOCIETAL IMPACT

To evaluate the impact of modern biotechnology on society, it is not enough only to measure it by specific indicators. When the focus is on such dimensions like increase the number of patents on genetically modified organisms (GMOs), this will not indicate the dynamic forces shaping the impact of biotechnology on industrial and societal structures. The impact analysis is combining complex problems and has a variety of diverse inputs, which possess both short- and long-term effects and influence the results of the problem or use of technology, in an unrespecting way. To realize the sense about the nature of links between societies, individuals, and the balance of forces between social groups facing new technologies is of basic importance.



ESTABLISHMENT AND SPREADING OF KNOWLEDGE

A starting point is the result of the technological impact, which depends upon the establishment and spreading of knowledge. It is regarded as preferred values, social practices, and norms of behavior of involved actors in the development and use of biotechnology. The knowledge extends beyond the collection of information on biotechnology, such as techniques, risks, etc. It also embraces the way they are shown and commented on by individuals. Risk and safety for the public cannot be assayed in the same manner as scientific risk is done. To account for these differences, it is necessary to explain how trust is created between consumers and manufacturers. In brief, such a definition of knowledge gives a piece of analysis on the impact of biotechnology and more broadly, environmental policies and practices.

From a conceptual point of view, this approach could be treated as a knowledge-based discourse. It is useful considering the expression of the biotechnological impact in the sense of the educational, social, and political background. The environmental discourses of biotechnology knowledge, and more specifically, how it is constructed, accepted, and perhaps most importantly, spread and sustained within society are taken into account as well.

SCIENCE AND TECHNOLOGY (S&T) IMPACT ON SOCIETY

In the EU there is a discussion on biotechnology and especially concerning genetic engineering and impacts arising from this technology. This issue has to be addressed strongly linked to the result of social interactions. Therefore, the way of realization of these dynamic forces should account for the interrelations and the form of resulting complexity between actors.

Another point to estimate, are two major topics when examining potential technology impacts. These are i) the immediate impact of the use and development of the technology and ii) the technology transcending risks. That means dependence on the long-term application of the technology and how to achieve and sustain a dominant position in its impact upon society from education, political and social perspectives. This could be sustained generally through more concern about the long-term consequences of S&T activities noted below:

- *Publication of educational efforts in society:* Performance of intensive efforts to include scientists, key public opinion leaders, and media in an existing debate on biotechnology and especially about genetic engineering. The core objective is to avoid an informational vacuum, which can facilitate anti-biotechnology activity and generate negativism in knowledge structures.

- *Role of regulatory policy in society:* Focusing on effective risk communication and understanding the fact that the public perception of risk can be very different from that of the scientific community.

PERCEPTION OF MODERN BIOTECHNOLOGY APPLICATIONS

Three major topics are identified and based around: i) the science, ethics, and gender of biotechnology ii) the International political economy, trade, and the environment; iii) the state and system security and warfare through biotechnology. These general themes are coming from consideration for the international relations of biotechnology. They imply the concept of power in the International Political Economy (IPE) to frame the differing discourses and complex issues of technology impacts. Based on these topics the several indicators are regarded (Fig. 3):

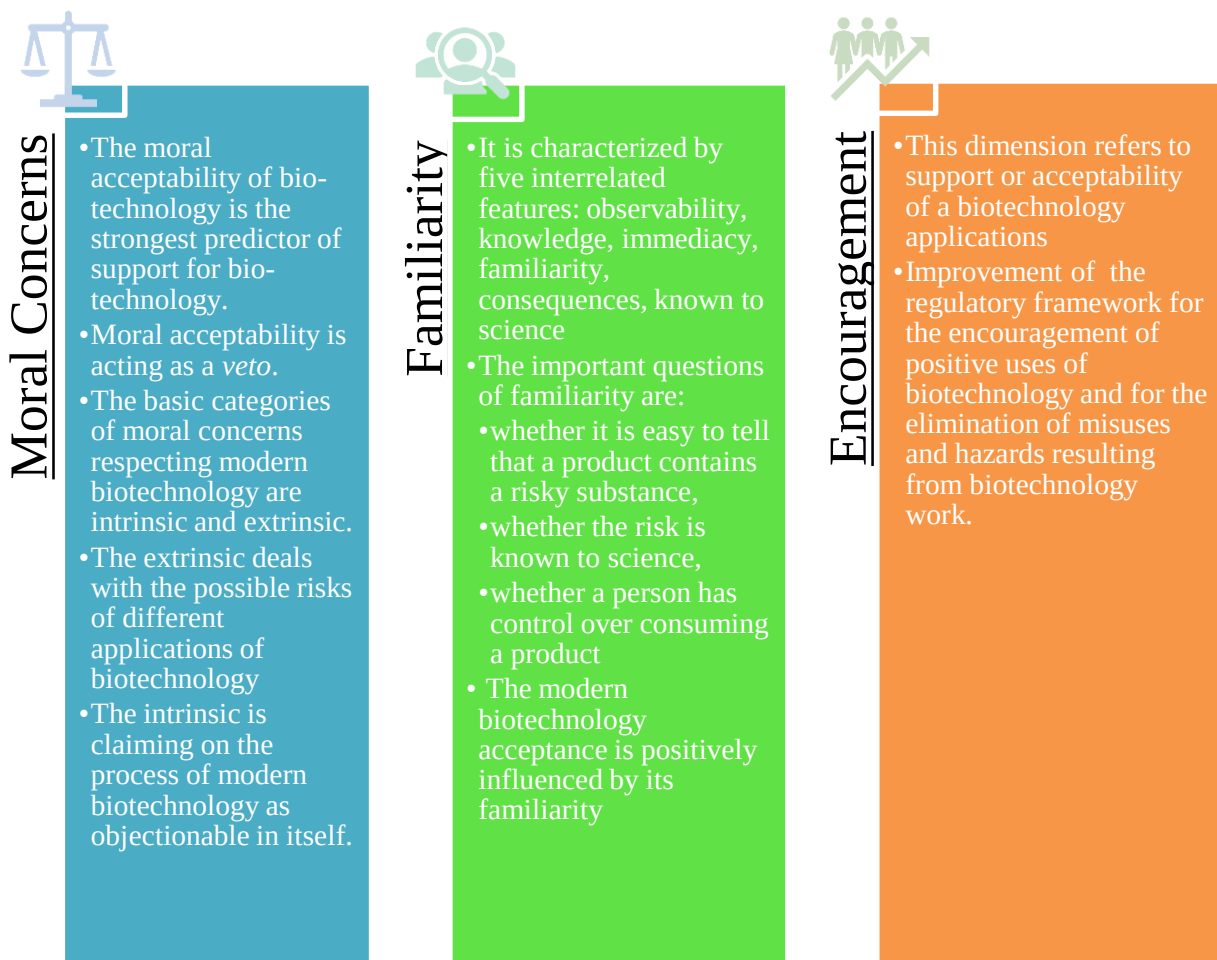


Figure 3. Social attitude indicators of modern biotechnology

Modern biotechnology is regarded as a complex emerging field that exhibits high science combined with limited knowledge of part of the society. It is related to attitudes towards the natural environment, technological progress, religious and moral beliefs, and several other sets. A matrix of variables including interest of the public domains, such as science and politics, optimism about technologies, social and cultural values, engagement with the issue of biotechnology and confidence in the industry, regulation, and other civil society groups, all these factors contribute to the public's representation of and opinions about biotechnologies (Fig. 4). Background characteristics such as gender, education, age, and religion have been found to affect people's attitudes as well.

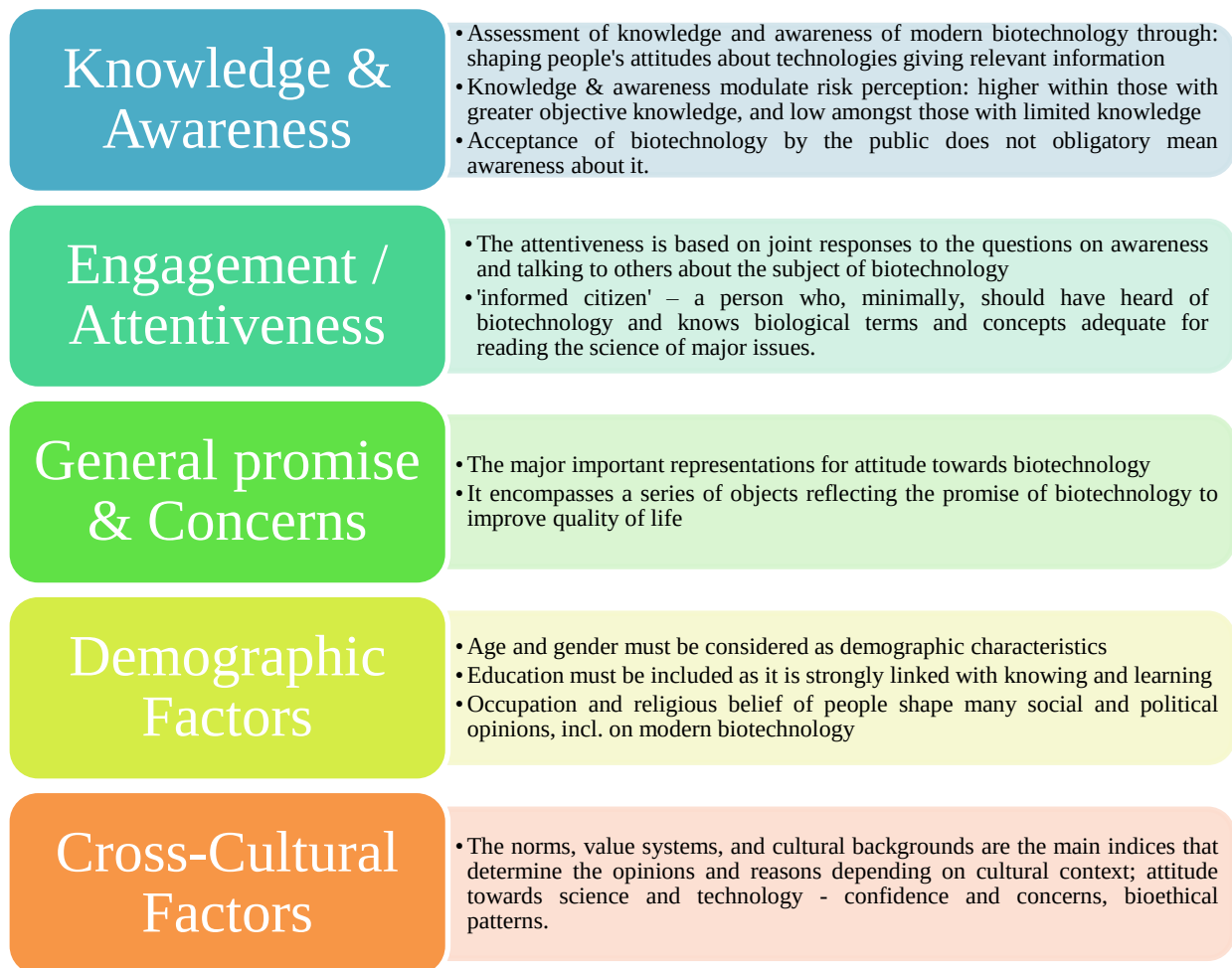


Figure 4. Factors of general attitude



2019-1-BG01-KA203-062371

The serious debate on the acceptance of genetic engineering is accompanied by many pieces of evidence that object to the focus on specific applications of the technology than genetic engineering *per se*. Studies are showing that consumer attitudes regarding gene transfers are influenced by the type of transfers. The range of acceptance encompasses the following: i) plant to plant gene transfers; ii) animal to animal transfer; iii) animal-plant or human-animal, the last being least acceptable.

A survey on perception on modern biotechnology applications, performed among people from different countries/continents indicated that medical applications (leading to the development of medicines and vaccines, and genetic testing) are very much acceptable in comparison to food or crop biotechnology applications. However, medical applications linked to xenotransplantation or animal cloning for milk production meet serious problems. The Asian people, for instance, are less troubled for medical genetically engineered products than for genetically modified food. The investigation concerning cloning of human cells and bioremediation among the Europeans is put at the intermediate level, less assumed than genetic testing but better confessed acceptable than genetically modified crop and food.

Another survey reported that genetic testing still scored the highest support within the European population, followed by human cell cloning, the production of GM enzymes for environmentally friendly soaps, and xenotransplantation. At the bottom of this range are placed GM crops and food. Also, higher support is given to technics for the manipulation of bacteria to clean oil spills followed by disease-resistant crops; afterward are applications for fat meat and better taste tomatoes and the less support receive technologies for enhancing milk production in cows.

The Malaysian consumers are more open for use of modern biotechnology applications, which do not involve inter-species gene transfers, like food production and GM crop that only involve the transfer of plant genes. For them, the gene modifications are more acceptable in the following order: i) transfer oil palm genes to reduce its fat (saturated) content, ii) transfer human genes into bacteria to produce insulin, iii) transfer bacterial genes into soybean to make it resistant to herbicides.

An individual's attitude towards a new technology depends on several related factors such as his/her perception of its risks and benefits, and socially communicated values and trusts in institutions representing these technologies. Concerning public perception of biotechnology, it is speculated that the attitude to genetic engineering is determined by the worth of potential benefits offered, knowledge on genetic engineering, and scientific world-view, from which the perceived risk (rational worries) and anxieties or fears (irrational worries) must be excluded. Additionally, various minor factors such as background factors must be added. It is considered that the main effect on acceptance seemingly is based on the knowledge level, awareness of benefits, confidence, and trust.

The studies of public attitude towards biotechnology indicate a lot of similarities with risk perception studies. Some authors have used the psychometric approach based on cognitive psychology and it is accepted as the most mature and dominant paradigm in risk perception studies. These psychometric methods are settled on the ground of the following views:



2019-1-BG01-KA203-062371

- To perform “risk” as a subjective idea, and not an objective one;
- To involve technical/physical and social/psychological outlooks in risk criteria;
- To take opinions of the “public” (i.e., laypeople, not experts) as the subject of interest.

Thus, the public acceptance of modern biotechnology is based on analyzing the cognitive structure of risk judgments, commonly using multivariate statistical procedures like factor analysis, multi-dimensional scaling, and multiple regression. This psychometric approach assumes that the public does not perceive technological risk due to a single dimension linked to expected injuries or fatalities close to a risk assessor's viewpoint but interprets risk as a multidimensional concept, concerned with broader qualitative dimensions.

The key varieties of a risk perception study are the perceived magnitude of risk or dread, risk acceptance, familiarity with the hazard, and at least - the factor benefit. The importance of another dimension, ‘interference with nature’ in risk perception studies on genetic engineering is also very important.

Biotechnology is at the confluence of science and ethics. The development of technology is linked with an ethical vision, which in turn is shaped by specific issues. Loads of biotechnology can be evaluated for their benefit to human society. But it has to be considered that biotechnology has also a dangerous part. It can provoke unanticipated consequences, which can cause harmful effects or dehumanize people. So, the ethical issues of proposed effects must be carefully investigated.

The ethical evaluation of new technologies, including biotechnology, requires a different approach to ethics. There is a necessity for changes because a new technology can have a more profound impact on the world due to i) restrictions to a rights-based ethics approach; ii) the importance and difficulty of predicting consequences; iii) opportunity now to manipulate humans themselves.

The ethical questions concerning biotechnology are very different. Due to the potential for deep change in the human future, such questions must be carefully considered. In the first place, it is necessary to articulate and predict the responsibilities towards nature and others, including future generations, and then to focus on rights and freedom. The real power and potential of biotechnology require strong caution to ensure ethical progress, as biotechnology is accepted as a significant force to improve the quality of people's lives in the 21st century.

Biotechnology is intrinsically linked to science and scientific knowledge. But sometimes there is doubt that biotechnology is closely tied to ethics. Lastly, different biotechnological trends are promoting a certain vision of life, some of which are good for life and deserved to be encouraged or pursued but others are bad and should be eliminated. This vision impacts people's choices and influences their sense of ethically appropriate biotechnology.

At times, the link between biotechnology and ethics is described as a conflicting point. Once, there is an impression that ethics is needed only in case one wants to tell others that they are doing wrong



things. To a certain degree, this is lucid, since dispute, debate, and argument are common parts of ethics consultation. But ethics is just as important in the case of consensus that the chosen pathway is good and right. Of course, there isn't any necessity for an ethical debate when the search is a cure for cancer. Thus, the solution to perform such a search is predicted by a common understanding that to cure cancer there is an ethical reason. The efforts, resources, and creativity devoted to working out better treatments are ethically eligible and the majority of these achievements due to biotechnology.

Benefits and Risks from Biotechnology Applications

The societal acceptability of benefits and risks imposed by modern biotech applications concern:

- Benefits vs. risks societal dispute
- Risk acceptance as a key element in risk perception
- Tools for measuring public attitudes towards risks and benefits: risk management

BENEFITS VS. RISKS SOCIETAL DISPUTE

To determine the acceptability of biotechnological applications it is very important to evaluate the perceived benefits and risks. The diagram on Fig. 5 summarizes them.



Figure 5. Perceived benefits and perceived risks

RISK ACCEPTANCE

Another key variable is risk acceptance which is important to measure risk perception. But it is rarely used in attitude towards biotechnology studies. Highlighted the harsh situation with modern technologies, they are always linked with some kind of risks that pose serious problems for societies. Policy-makers have considered cost-benefit analysis as the basis of decision-making methodology for societal risk acceptance. The core question to be solved in the risk-benefit analysis is: whether this

2019-1-BG01-KA203-062371

product (activity, technology) is eligible safe or how safe is safe enough? There are two main approaches for performance of risk-benefit analysis (Fig. 6).

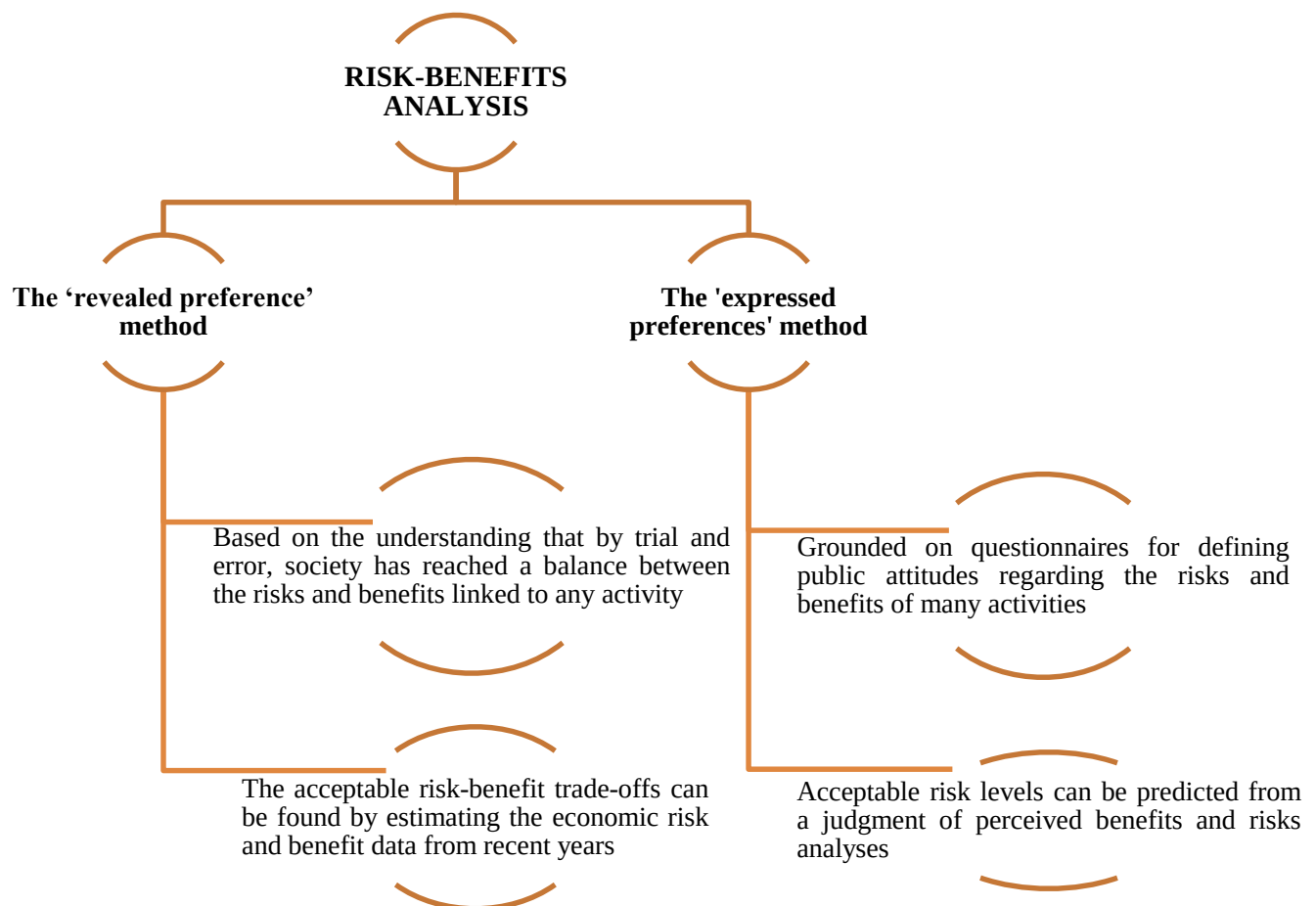


Figure 6. Approaches for risk-benefit analysis



RISK MANAGEMENT

The management of risk is the process that aims to diminish the effect and to balance the way people respond to it. If one wants to push away all risks, this is not a viable goal. People readily take risks performing a wide range of activities. Thus, the question about the acceptable risk is not entirely technical; its answer depends on values. In a simplified way, it is possible to set a simple threshold for acceptable risk, for instance - one in a million-lifetime risk. All the activities riskier than this threshold are “unacceptable” as well as all those less risky are “acceptable”.

However, there are two reasons why this approach is not working. The first is linked to the above-mentioned risk perception. The people are not reacting to risk simply; the acceptability of risk in the public's mind is not simply a matter of the presumable fatal things but involves other elements of the activity. For instance, if the activities are taken voluntarily if the process leading to the risk is new/old or familiar, if the process provokes an intuitive, emotional dread reaction or not. That is why, to define an acceptable risk, the processes of risk communication and engagement described above must be included. In the second issue, some activities are essential and must be encountered even if they cause relatively high risks.

Besides, there could be other cases where inexpensive alternatives exist to perform less risky or modified activities. In these cases, the risk might be well predicted as unacceptable, due to ready alternatives. This is an economic issue that deals with several resources dedicated to risk reduction. Besides the economic problems, it is possible to consider risks, not in terms of absolute values but to their internal differences between alternatives.

However, very often it is not possible fully to escape risk; rather one must choose among different risks. Therefore, no definite acceptable threshold of allowable risk exists, as well as there is no single convenient monetized value for risk diminution. Societal desire to invest in risk reduction depends on the wide range of factors that manage risk perception, like the fear associated with the risk, its catastrophic potential, etc.

SOCIAL BENEFITS OF MODERN BIOTECHNOLOGY

Biotechnologies are being used widely in such fields as medicine and hygiene, agriculture, forestry, breeding, fisheries, energy and chemical industries, metallurgical and mining industries, food, and light industries, environmental protection. Modern biotechnology has gradually demonstrated its huge potential contribution to productivity, though it has a short history of only tens of years so far. Modern biotechnology will become powerful means to reduce the world's constraints in the fields of food, health, energy, resources, and the environment. The development and application of modern biotechnology will, over a long time, affect human beings and society deeply.



Biotechnological industries are likely to become the leading industries in the 21st century. For example, crop varieties, produced by biotechnology, with novel traits of high productivity and stress resistance (aridity, coldness, high salt, etc.) are expected not only to increase grain production but also decrease the use of agricultural chemicals (such as pesticides, herbicides, and fertilizers), which will be beneficial to the environment. In the respect of medicine and hygiene, many drugs such as DNA vaccines, protein engineering medicine, monoclonal antibodies, anti-sense RNA drugs have been developed by biotechnology. Transgenic animals and plants producing some kinds of medicines (such as vaccines and hormones) may help to reduce illness in the process of everyday diet. Human Genome Project (HGP) and the research into some disease genes will discover the genetic reasons for some illnesses. Environmental pollution is getting worse at present, but biological transformation reactors created by biotechnology promise to absorb pollutants or wastes and decompose them into materials of low or no toxicity.

Therefore, in the present society characterized by knowledge-intensive industries, countries all over the world, especially the industrialized ones, take biotechnology seriously. Large quantities of manpower and financial resources are allocated to R & D in biotechnology. Relevant development strategies and policies are drawn up and support mechanisms created to stimulate the development of biotechnology.

Acceptance and diffusion of modern biotechnology

Although there have been many studies on public attitude or perception towards biotechnology and some researchers tried to identify factors predicting attitude using either regression or correlation, there are limited studies that try to construct a structural model predicting attitude towards biotechnology.

The first documented model was developed by Kelley (1995) who proposed a structured way for the approval of genetic engineering by the Australians (Fig. 7). Later, Pardo et al. (2002) proposed another model for explanation of European attitude towards biotechnology applications. Kelley found out that approval of genetic engineering was mainly predicted by agricultural and health goals (beneficial aspects). On the other hand, scientific and genetic engineering knowledge did not predict approval. Demographic variables also did not directly affect the approval of genetic engineering (their effects were not statistically significant). Gender was found to affect knowledge and scientific world-view while age was found to affect knowledge, goal, and scientific world-view, thereby exerting a very weak (indirect) effect on approval. Education had a large effect on knowledge but not concerning attitudes. Occupation and religion did not have any effect on any of the intermediate variables. Green supporters were less

favorable to genetic engineering (negative direct effect on the approval of genetic engineering) as compared to Labor Coalition supporters (politic).

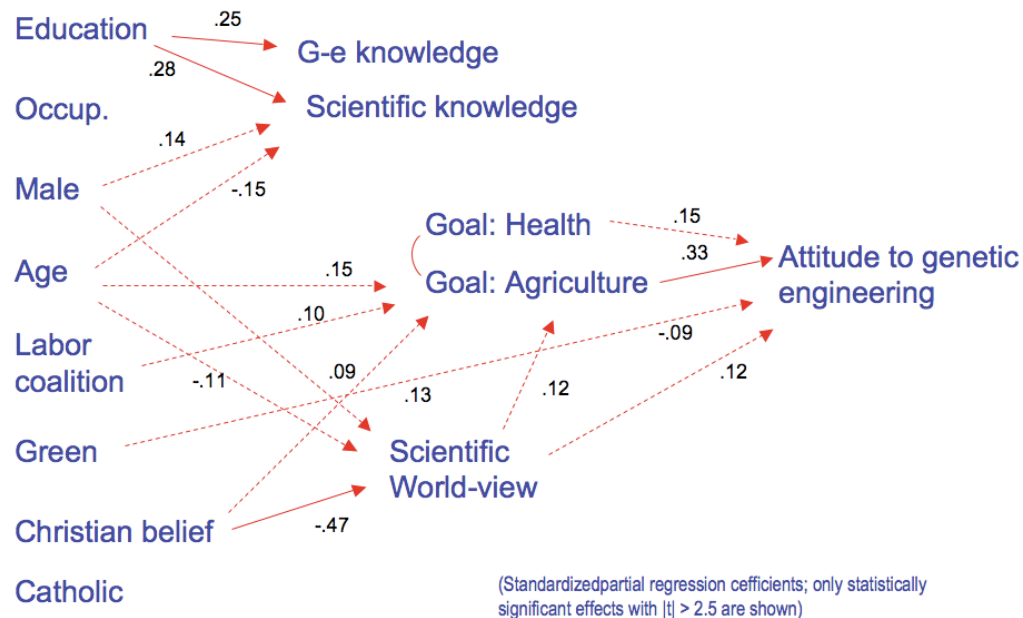


Figure 7. An ‘Attitude towards the genetic engineering’ model of Kelly (1995)

It is often speculated that biotechnology is the technology to deeply transform the economy and society. Following such opinions, the development and application of biotechnology have considerable potential for far-reaching economic, social, and environmental impacts. That is why biotechnology is of strategic importance to knowledge-based economies and their governments. Nations that failed to develop biotechnology capabilities fail in realizing economic impacts.

Biotechnology-related applications are diffused over several very different industrial sectors, e.g., food production, textile finishing, pulp and paper, agriculture, power generation, chemicals and petrochemicals, and pharmaceuticals. The products manufactured by these sectors do not usually distinguish between alternative production processes. Thus, for many products, a distinction between biotech and conventional (e.g., chemical) production processes and also the introduction of some new ones would be necessary. Moreover, biotechnology is a dynamic field and certain biotechnological production methods will probably soon be re-developed, and completely new, products will appear. Current official statistics and existing companies' surveys can provide specialized information about the diffusion of biotechnological applications, and their economic impact. The data have been constantly renovated and different techno-economic studies are performed to provide future production foresight



2019-1-BG01-KA203-062371

and scenarios. Applying a blend of methods and sources, they frequently focus on expert judgment, case-studies, and various statistics.

An alternative to measuring the diffusion of biotechnology in use for major manufacturing sectors is the biotechnology-related-sales (BRS) concept. Existing studies have used various approaches for measurement (definition of the biotechnology aggregation level, examined sectors, scenario assumptions, etc.). Moreover, to assess the impact of biotechnology diffusion, an input-output model and relevant indicators have been introduced to study its effect on employment. The model studies in a comparative manner how biotechnology is affecting major application sectors. It compares the market penetration in 2004 and 2020 through the diffusion rates, measured with upper and lower limits. This approach helps not to overestimate the impact of biotechnology while capturing certain innovative biotechnological applications. The data of this survey indicate a nearly similar diffusion pattern in pharmaceuticals, food-processing, and agriculture. Diffusion is expected to increase in all sectors greatly by 2025, as indicated by the penetration of certain product groups. However, it was shown that there is a serious level of suspicion about the diffusion of biotechnology; the expectations for 2025 are highly uncertain. The analysis of this tendency, indicate that there are diffusion barriers specified in Fig. 8.

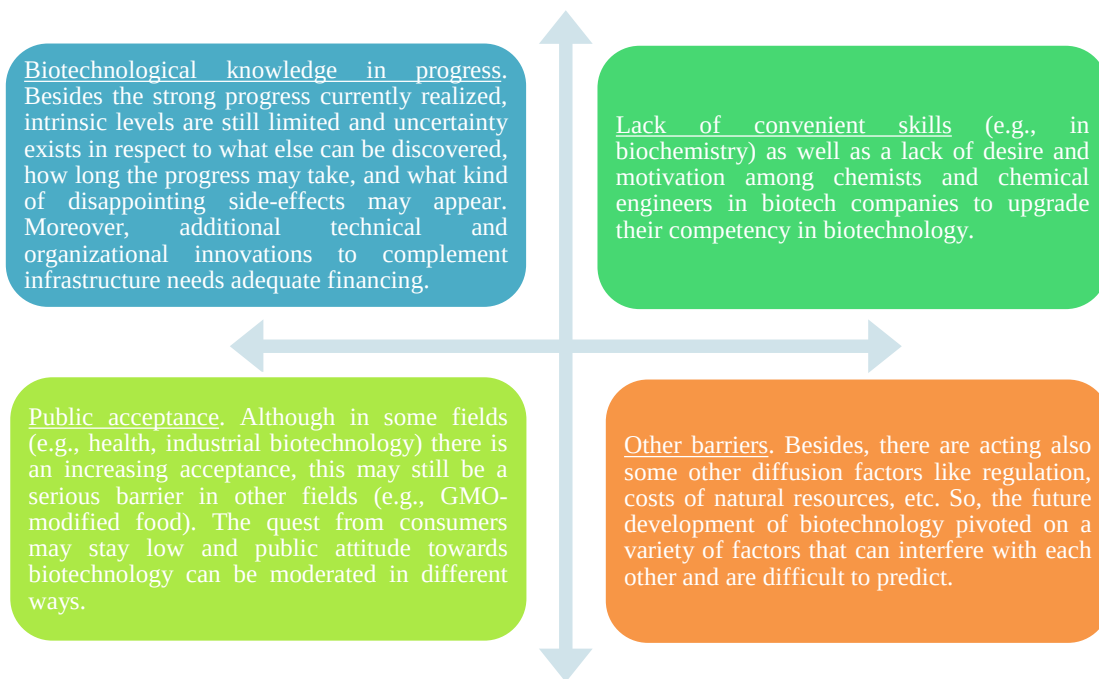


Figure 8. Main barriers interfering Biotechnology diffusion



Possible negative impacts of modern biotechnology

SOCIOECONOMICAL IMPACTS

Motivated to perceive great economic profit, a lot of famous international business corporations made considerable investments of capital in the R & D of biotechnology, and in this way are changing the previous world's social and economic trends. Such an example is the company Monsanto (formerly US-based, and now a part of the global consortium BASF). Traditionally working in the chemical industry, after 1985 the company made serious business rearrangements, invested in 3 biotechnological sectors, and became a major consortium dealing with R & D in bio-science. After 1998, about 20 million hectares of fields all over the world were planted with Monsanto's GM seeds. Soon after that, Monsanto took an advance on the crop seeds international market through a completely integrated system for trading GM seeds and agricultural chemicals. In this way, Monsanto received control on a big part of the human population food chain gaining tremendous profit feeding them with GM crop. This is an example of how in the current globalized world, a possibility for a few multinational corporations to decide on human food consumption emerges.

In the case of use in dairy, bovine growth hormone (BGH) produced by genetic engineering, the milk yield of a dairy cow can increase by 30 percent., which means 10 percent less fodder usage. So, the result is very impressive for the dairy industry.

A lot of medicines, like vaccines for treating malaria and cholera are provided to patients via new antigens synthesized through modern biotech methods. They make the rates of illness and death in many countries (especially in the developing ones) falling rapidly, concurring the population growth rates. In this context, a question arises about the impacts on the already insufficient economics and infrastructures of the said countries.

IMPACTS ON BIODIVERSITY AND SUSTAINABLE AGRICULTURE

Many GM crops contain foreign genes from other plants, animals, and microbes. All these foreign genes can be transferred to other plants in nature through the pollens of genetically transformed plants and will cause pollution to the natural pool of genetic resources. Hence, as the majority of transgenic plants possessed economically useful features, like high productivity, disease-pest resistance, stress resistance, and farmers are attracted to economic interests to grow these plants rather than traditional crop varieties, the predicted danger for the natural resources is serious. Also, the technologies for breeding of a monoculture of crop varieties in some fields may add to the loss of biodiversity. So, in a long term, this degradation of the genetic base may reduce the natural resistance of plants to diseases and insect pests and this may cause diminish in the crop yields. In this way, the global sustainable development could be compromised.



2019-1-BG01-KA203-062371

Bacillus thuringiensis (Bt) is a soil bacterium, which is used to synthesize a toxin against insects within plants. It has been used as a microbial pesticide for many years and the genes coding these insecticidal toxins could be introduced in the cell of many crops such as cotton, soybean, and rape, to create pest-resistance crops. In this case, the experts in environmental protection and the Green Peace Corporation are troubled by the fact that the toxin production is realized in the late life cycle of the transgenic plants and there is an opportunity to increase commonly the resistance of pests to the Bt toxins. So, this situation would lead to bigger usage of Bt-based pesticides, due to loss of their efficacy and causing heavy economic losses.

IMPACTS ON PUBLIC WELFARE

The exploitation of genetic engineering technology to humans could facilitate the diagnose and cure of genetically related diseases such as some cancers, hemophilia, etc. But such gene diagnosis may also have an opposing effect on the employment and marriage prospects of human beings. Through genetic engineering animals, plants and microbes can be transformed into bio-factories for the production of different kinds of chemicals. Thus, they can be treated as constant biological producers and at the same time, the rising potential for products outcome can cause socioeconomic problems. Assume that specific microbial strains are engineered to produce a valuable substance on large scale. In such a case, it is important to forecast the impact of this production on the world's major producers of the same substance. If this substance counts for a significant percentage of the income of a given country, what would be the influence and consequences of this biotech production on the country's agriculture and financial infrastructure.

Biosafety issues of modern biotechnology

Inconvenient application of biotechnology can cause many risks, like other advanced technologies. For instance, the newly introduced genes in crops made by genetic engineering can lead to allergic reactions. The economically important features of pest-resistance, herbicide-resistance, or stress-resistance, introduced in plants may get these transgenic hosts away from agricultural cultivation systems. The genes for resistance in transgenic plants can be transferred to their wild weedy relatives, and the latter can be transformed into "super" weeds, which control will be very difficult. Thus, the large-scale releases of transgenic pest-resistance crops into the environment will cause severe selection pressures to enforce the resistance of target pests.

The transgenic virus-resistance plants that are already transformed with foreign virus genes possess virus-coded proteins that may recombine with the genetic material of other viruses to yield new types of viruses with higher toxicity. If the selective killing of the target pests and pathogens is not



2019-1-BG01-KA203-062371

possible, the transgenic pest-and-disease resistance crops can at once poison other organisms and also may cause harmful action via food chains on beneficial microorganisms, insects, birds, and mammals.

Moreover, many biotechnological products are alive organisms and can move and reproduce by themselves. Once they are released into the environment and are proven to be toxic, then it is nearly impossible to discard them, and the harm they create can rise with time becoming more and more serious. In this way, environmental releases of transgenic plants on a large-scale may damage the natural ecological balance in a longtime perspective.

Biosafety concerns have hindered the R & D applications of biotechnology. The public in lots of countries has signified its negative opinion, repulsion, indeed dread regarding biotechnological products. This is voiced even through demonstrations, crushing fields crops, banding the import, or buying the biotechnological products. This makes the biosafety issues at the very top of the agenda of many countries and international societies. Laws and regulations on biosafety are formulated in many countries, and biosafety issues are regarded in many international documents and treaties, like Agenda 21 and The Convention on Biological Diversity.

Conclusions

Biotechnology is on the way to become seriously relevant to the economy and can gain considerable importance in several application sectors in the next 10 to 15 years. However, the overall effects of modern biotechnology on the economy are quite diverse. For instance, employment estimations show that increasing the part of biotechnology in application sectors and its link to suppliers indicate the trend for reduction of employment due to productivity effects. The economic importance of biotechnology rises because of its diffusion in mature manufacturing sectors. However, diffusion in these main application sectors can influence only limited parts of overall employment. Only if biotechnology increases in other sectors might a major economy-wide impact emerge.

The deepness of biotechnological transformation of the economy depends strongly on its potential to propose the creation of new products and to improve the efficiency in the production of existing ones. Hence, it is expected that biotechnology would enable the creation of many products but the potential to raise labor productivity is estimated to be restricted. One important view is that biotechnology may transform the economy and society differently, cooperating with other technologies like ICT. Major boosts for such economic growth may emerge in combination with other technologies or efficiency gains, which raise productivity and purchasing power to realize the fruits of biotechnology.



References

Agreements on Trade-Related Aspects of Intellectual Property Rights (TRIPS) available from https://www.wto.org/english/docs_e/legal_e/27-trips.pdf

Amin L, Hashim H, Sidik NM, Zainol ZA, Anua N. 2011. Public attitude towards modern biotechnology. *African Journal of Biotechnology* Vol. 10(58), pp. 12409-12417, Available online at <http://www.academicjournals.org/AJB> DOI: 10.5897/AJB11.1061

Amin L. 2013. *Modern Biotechnology in Malaysia* (UM Press)

Biology Online. Blue Biotechnology. http://www.biologyonline.org/kb/biology_articles/biotechnology/blue_biotechnology.html

Gaskell G, Alum N, Baouer M, Durant J, Allansdottir A, Bonfadelli H, Boy D, Cheveigne DS, Fjaestad B, Gutteling JM, Hampel J, Jelsoe E, Jesuino JG, Kohring M, Kronberger N, Midden Holden C. 1990. Activists urge ban on herbicide R & D. *Science*, 247:1539.

Horst W, Doelle J, Rokem S, Berovic M. 2019. *Biotechnology - Volume XIII: Fundamentals in Biotechnology*, EOLSS Publications, p 364.

ISAAA & The University of Illinois at Urbana Champaign (UIUC). 2003. *The social and cultural dimensions of Agricultural Biotechnology in Southeast Asia Report*.

Kelley J. 1995. *Public perceptions of genetic engineering: Australia, Final report to the Department of Industry, Science and Technology*, May 1995.

Lorenz P, Zinke H. 2005. White Biotechnology: Differences in US and EU approaches? *Trends. Biotechnol.* 23(12): 570-574.

Luscombe N, Greenbaum D, Gerstein M. 2001. What is Bioinformatics? A Proposed Definition and Overview of the Field. *Method. Inform. Med.* 4: 346-358.

Miller H, Huttner S. 1998. A baroque solution to nonproblem. *Nature biotechnology*, 16: 698-699.

Nielsen TH, Przystalski A, Rusanen T, Sakellaris G, Torgersen H, Twardowski T, Wagner W. 2000. Biotechnology and the European public. *Nat. Biotechnol.* (18): 935-938.

O'Mathúna DP. 2007. Bioethics and biotechnology. *Cytotechnology*. 53(1-3): 113–119. doi: 10.1007/s10616-007-9053-8



2019-1-BG01-KA203-062371

Pardo R, Midden C, Miller JD. 2002. Attitudes toward biotechnology in the European Union. *Journal of Biotechnology* 98, 9-24.

Wydra S, Nusser M. 2011. Diffusion and economic impacts of biotechnology – a case study for Germany. *Int. J. Biotechnology*, Vol. 12, Nos. 1/2, 2011 87 DOI: 10.1504/IJBT.2011.042683.



Project website: www.digit-biotech.eu

The European Commission's support for the production of this publication does not constitute an endorsement of the contents, which reflect the views only of the authors, and the Commission cannot be held responsible for any use which may be made of the information contained therein.

DIGIT~Bio~TECH



LO8 BIOETHICS AND MODERN BIOTECHNOLOGY

Advanced level

AUTHOR:
JÜRGEN SIMON



Contents

Synthetic Biology and Cloning	3
Introduction	3
1. What “Synthetic Biology” means	3
2. Safety issues in synthetic biology	6
3. Sustainable Models for the Protection and Use of Intellectual Property.....	9
4. Challenges of the foreseeable new genetic engineering debate.	10
5. Overall assessment	11
Cloning of Animals and Humans	12
1. Introduction	12
2. Biomedical research and application.....	12
3. Legal aspects	14
4. Ethical aspects	15
5. Conclusions and options for action	15
Law and Ethics in the Field of Environmental Sustainability	16
Introduction	16
Europe's commitment to sustainable development	16
Regulation of environmental behaviour.....	17
Instruments.....	18
1. Instruments to enforce the environmental policy (environmental planning)	18
2. Instruments to regulate the environmental behaviour	19
3. Principles for political and legal measures.....	19
Regulation of Environmental Behaviour.....	23
1. Instruments to enforce environmental policy (planning)	23
2. Instruments to regulate environmental behavior	24
The Multidimensional Sustainability Strategy	26
Equal opportunities as a universal ethic of globally integrative sustainability	28
References	30



Synthetic Biology and Cloning

INTRODUCTION

Synthetic biology is a recent extension of biotechnology in which genes and proteins are viewed as parts or devices, with the goal of rearranging and/or assembling these parts in novel ways to create new and useful functionality. Recent advances in biofuel generation, biochemical production, and minimal genome understanding all benefit from synthetic biology approaches. Often, these projects rely on the ordered assembly of multiple DNA sequences to create large, artificial DNA structures. To this end, methods have evolved to simplify this process.

Synthetic biology combines molecular biology and systems biology with engineering principles to design biological systems and biofactories. The goal is to create improved biological functions to address current and future challenges.

1. What “Synthetic Biology” means

For well over a decade, the term "synthetic biology" (Synbio for short) has been used to describe research projects, methods, and procedures to "rebuild natural organisms." This goes further than was previously possible with the help of genetic engineering. The approaches extend to the creation of (complete) artificial "biological" systems. The short- and medium-term significance as well as the longer-term potential of this very heterogeneous field are assessed quite differently within science, industry and politics, which is also due to the still lacking stringent definition.

The basic distinction of synbio in the narrower and synbio in the broader sense is made and used for the impact analysis and debate:

Synbio in the narrow sense refers to the production of cells or organisms (or cell-free biological or biochemical systems) designed "on the drawing board" and constructed de novo. These are intended to be used for the production of any, even completely novel substances or visionary applications in the fields of health, energy or the environment. Characteristic research approaches and methods

- (1.) the production of complete synthetic genomes,
- (2.) the construction of so-called "minimal cells" (either "top down" by reducing natural cells or "bottom up" or "from the scratch" from basic biochemical components), and



2019-1-BG01-KA203-062371

(3.) the use of non-natural molecules ("xenobiology").

Synbio in the broader sense, on the other hand, is a collective term for all currently pursued, increasingly information-based and mostly application-oriented approaches to molecular biological modification of known organisms. These aim at the construction of new synthetic pathways for the production of chemicals or the design of genetic circuits for new sensory and regulatory functions in existing organisms. Synbio in a broader sense goes beyond previous simple genetic engineering approaches to influence the metabolism of organisms (so-called "metabolic engineering"). Increasingly, computer-aided design and modeling processes are being used.

Synbio in the broader sense also includes genome editing processes, which have hardly been covered under the Synbio label so far. In spring 2015, their rapid development and possible application to plants, animals and also humans provided the impetus for an intensification of the genetic engineering debate at both international and national level, which will also include synbio as a research area and funding object.

In other words, synbio brings together various scientific disciplines such as molecular biology, organic chemistry, nanotechnology, information sciences, and areas of medicine to purposefully modify biological organisms, combine them with artificial elements, or create completely artificial organisms ("artificial life" or "ALife").

It is described as one of the newest and most promising developments in modern biology. It is part of the new and emerging science and technology (NEST). So far, no unified scientific - and therefore even more so no legal - definition has been found. Ethical, theological and legal challenges related to synbio are widely discussed in view of its implementation orientation, the enormous scientific progress and the considerable (concrete) application potential of synthetic biology.

Five subgroups are defined as the main application areas of Synbio:

- a) DNA synthesis: chemical construction of genetic codes based on the matrix of a genetic code of an existing organism (with known nucleic acids).
- b) DNA-based biological circuits: Transfer of complete biological systems from biobricks.
- c) Minimal genome or minimal life form (top-down process)
- d) Protocells: living cells that are re-engineered from the bottom-up
- e) Xenobiology: creation of orthogonal biological systems not found in nature, based on biochemical principles not found in nature (XNA).

These five subgroups can be reduced to three main elements:

- 1. modification,
- 2. copying and
- 3. new creation of "life".



2019-1-BG01-KA203-062371

The only regulatory gap that could be identified concerns the element of "new creation"; thus, the question arises whether also a "de novo" synthetically created cell or an orthogonal biological system that does not occur in nature is a "biological entity capable of reproducing or exchanging genetic material" within the meaning of the GenTG. The Central Commission for Biological Safety (ZKBS) in Germany stated in its current interim report of 06.11.2012 that most scientific approaches to synthetic biology fall within the scope of the GenTG. Only novel living systems such as artificial cells (bottom-up approach) without a model in nature are not covered by the GenTG. In this respect, a small clarifying addition to the legal definition of the term organism in the GenTG would be sufficient to close the gap. The addition could be worded as follows: "any biological entity capable of reproducing or transferring genetic material, including microorganisms, as well as any biological entity created by technical means that does not occur under natural conditions and that contains genetic material that does not occur in nature." A corresponding amendment would clarify that synthetically produced or modified organisms or biological entities and even the use of naked, synthetically produced DNA would definitely fall within the scope and control of the GenTG.

Although synthetic biology does not appear to be a fundamentally new technology - especially in the legal sense - but more or less a direct continuation of modern molecular biology, genetic research or genetic engineering, the question arises whether the existing laws are sufficient or whether new laws are necessary, given the considerable (concrete) potential for application of synthetic biology.

In its most recent decision on the GenTG, the Federal Constitutional Court clarified that the legislature has a special duty of care in assessing the long-term consequences of genetic engineering because the state of scientific knowledge is not yet complete. In this respect, the mandate of Article 20a of the German Basic Law (GG) must be observed, which calls on the legislature to assume its responsibility for future generations by protecting the natural foundations of life. "This mandate calls for both hazard prevention and risk precaution. Among the environmental goods protected by Article 20a of the Basic Law are the preservation of biological diversity and the protection of a species-appropriate life for endangered animal and plant species". In this context, the Federal Constitutional Court has made it clear that the regulations of the GenTG are intended in particular to ensure protection against the uncontrolled spread of genetically modified organisms. However, the legislator must take into account new findings and new scientific knowledge and examine whether changes in the practice of risk assessment are necessary. If this is the case, the legislator must react accordingly and adapt the legislation. If the new level of risk exceeds the socially acceptable level of risk, the legislature must take action. Legislators have a duty to maintain a high, if not the highest possible, level of protection for human health. If they fail to comply with this duty, case law may ultimately find a violation of the precautionary principle.



2019-1-BG01-KA203-062371

2. Safety issues in synthetic biology

Questions of biological safety have accompanied the internal and external scientific debate on synbio from the outset. Since most Synbio products and processes are at the beginning of their development, their possible safety-relevant properties such as toxicity, allergenicity, dispersal behaviour or survivability are also largely unknown. In connection with the discussion about the nature and novelty of synbio, the safety debate on biosafety has for some time focused on the politically significant question or examination of whether the current and foreseeable developments (still) fall under the current regulations for medicinal products, advanced therapies, medical devices, chemicals and, above all, genetically modified organisms (GMOs) or are adequately covered by them - or whether the category boundaries are being blown up and previous risk assessment and risk management procedures are no longer effective. A second complex of topics concerns questions of biosecurity, i.e. the illegal (biocrime) or even malicious (bioterror) use of biological agents or the underlying knowledge. Even though much-discussed and controversial experiments (for example, bird flu viruses) that have been associated with the danger of such misuse have so far not primarily come from Synbio research projects. But scenarios of a future synthetic biology are associated with far-reaching fears and have already led to initial regulatory efforts.

BIOSAFETY ISSUES - CHALLENGES FOR RISK ASSESSMENT AND RISK REGULATION

A current need for a revision of risk regulation for GMOs in Germany and Europe, specifically with regard to "synthetically" modified organisms (SVOs), is still evident today. However, in view of the dynamics of scientific and technological development as well as the regulatory differences in various regions of the world, a forward-looking, more intensive consideration of the risk regulation of a possible future release of SVOs appears to be entirely appropriate.

The central issue for the risk assessment and risk-benefit evaluation of future SVOs is the question of how a safety assessment without substantial equivalence to a familiar parent organism would have to be conducted in such a way that the result could be accepted by actors in research, industry, politics as well as by civil society organisations and the public/citizens as a basis for approval for field application. In the case of plants, this question arises from a major genetic engineering "conversion" onwards; in the case of microorganisms, it basically arises with every type of field application, for example with an open microalgae culture for biofuel production, because these have so far been used almost exclusively in closed systems. Interventions in human intestinal and other microflora could become a highly explosive issue because the regulatory responsibilities are unclear here: The German Genetic Engineering Act (GenTG) does not refer to the application of genetic engineering to humans and thus probably not to the components of the human microbiome as long as they are in the human body.

The Joint Policy Paper of the German Research Foundation concludes that there is currently no need for action, or at least no significant need for action. The areas of conflict in synthetic biology are



2019-1-BG01-KA203-062371

covered by existing law and are thus sufficiently regulated. The German government comes to the same conclusion. Questions of biosafety are covered by the Genetic Engineering Act (GenTG), the Medicines Act (AMG), the Infection Protection Act (IFSG) and the Chemicals Act (ChemG). In the view of the German Research Foundation, these regulations are currently largely sufficient, so that there is no acute need for action. This is also the predominant view of the German authorities.

The German Ethics Council also sees no need for action, since synthetic biology in Germany falls entirely within the scope of the GenTG and aspects of biosafety are therefore largely irrelevant. The most important task at present is probably to develop a consistent definition of synthetic biology, to clearly distinguish it from other technologies and to formulate an answer to the question of what the essential novelty of this technology actually consists of. The Council also recognizes that the development of synthetic biology may create new problems and security risks that require a response or debate on how to respond. For this reason, the importance of some kind of monitoring process and its continuous improvement is emphasized. This monitoring process must be constantly improved. Monitoring is required by law. The ZKBS (Central Commission for Biological Safety) has already responded to the call for monitoring of synthetic biology and has submitted a first report on its observations/on the topic (1st Interim Report of the Central Commission for Biological Safety (ZKBS) of November 6, 2012, "Monitoring of Synthetic Biology in Germany"). In this report, the ZKBS, in accordance with an assessment and monitoring duty assigned to it (ZKBS 2012), examines several new techniques that belong to synthetic biology and concludes that they either fall within the scope of the GenTG or - if this is not the case - do not generate risks that require regulation. The same applies to cells generated de novo or to orthogonal biological systems.

An addition to the purpose of the law within the meaning of Section 1 GenTG is merely declaratory and thus not necessary. In its most recent decision on the GenTG, the Federal Constitutional Court clarified that the purpose of the regulations contained in the GenTG is, in particular, to ensure protection against the uncontrolled spread of genetically modified organisms.

In addition, Testbiotech demands that § 16 GenTG be supplemented as follows: "(2) A release of genetically modified or synthetically produced organisms shall be prohibited if their spread cannot be controlled or their retrieval cannot be ensured".

In principle, such a regulation is not objectionable from a constitutional perspective. The assessment of the risk of danger falls within the prerogative of the legislature and does not require scientific-empirical proof of the actual potential danger posed by genetically modified organisms and their progeny. In a situation that cannot be scientifically clarified, the legislature is entitled to assess the danger and risk, especially since the protected legal interests are constitutionally fixed and have a high value, and the existing risk of undesirable or harmful, perhaps even irreversible effects should be controlled in the sense of the greatest possible precaution. The Federal Constitutional Court also refers to Explanatory Memorandum No. 4 and No. 5 to Directive 2001/18/EC.

Ultimately, it will hardly be possible to provide conclusive evidence that the unintentional spread of genetically modified or synthetically produced organisms can be controlled and that their recovery / retrieval is guaranteed in any case. The addition to § 16 GenTG postulated by TestBiotech would not



2019-1-BG01-KA203-062371

only affect synthetic biology. Rather, it would establish a ban on the release of genetically modified organisms into the environment for all areas of genetic engineering, i.e. for all genetically modified organisms. A restriction that has been postulated since the first genetic engineering debates would thus be put into effect. Therefore, such a demand will hardly be politically enforceable. The demand for such a restrictive regulation regarding the release of GMOs gives rise to the assumption that the critics/opponents of genetic engineering will use the supposed novelty of synthetic biology to discuss and finally enforce their old demands for a limitation of genetic engineering. This would probably mean the end of synthetic biology and genetic engineering in Germany. The long-term study of environmental compatibility demanded by the EGE would also hardly be feasible/possible, because such a study would ultimately require the release of organisms. Only a controlled release of GMOs can provide "real" and comprehensive findings on environmental compatibility in the natural environment.

Another issue could become the renewed consideration of safety requirements for production organisms also in contained systems ("contained use"), especially with regard to possible "fully synthetic", largely newly engineered or xenobiologically massively modified organisms. Even though they are still far from being ready for use, they have been increasingly put up for discussion by some scientists as a supposedly particularly safe future option because of their fundamental biochemical differences, which, among other things, are supposed to make functional gene exchange with natural organisms impossible.

In all likelihood, the risk debate on genetically modified insects or animals in general will gain in importance in the coming years - especially due to the increasing possibilities of genome editing techniques. In view of the experience gained with the approval of transgenic plants, a consensual positive risk assessment of genetic engineering interventions in animals, especially those with a high potential for dissemination such as insects, seems very unlikely in the EU.

BIOSECURITY ISSUES - PROTECTION AGAINST MISUSE

Deliberate misuse of bioscientific findings can include not only the targeted development, production and transfer of biological weapons/combatants by regular military institutions or terrorist organizations, but also criminal activities such as the production of drugs, doping substances or counterfeit medicines. By their very nature, little is known about these either clandestine or illegal activities, which is why a detailed, fact-based debate to assess the dangers of "bioterror" and "biocrime" (as a result of synbio activities, but also otherwise) cannot actually be conducted publicly. However, questions can be raised in principle about the potential misuse of technologies that can be used both for societal good and deliberately for harmful purposes - so-called "dual-use technologies".

This involves two levels:

1. the generation of sensitive knowledge - e.g., for the synthesis and production of toxic substances, highly pathogenic viruses, or resistant bacterial pathogens - and



2019-1-BG01-KA203-062371

2. access to this knowledge and to the technologies or apparatus (laboratory equipment) necessary for its realization.

Control of the undesired proliferation of knowledge and technologies in the life sciences faces major technical, but also conceptual, legal and ethical challenges. The latter are rooted in questions about the restriction of the constitutionally protected freedom of research as well as concrete, potentially important possibilities for health research and health care; but also in questions about whether and how knowledge can be selectively passed on to selected groups and who could or should decide about this knowledge and the selection of those "entitled to receive" it. There is consensus that, in addition to international arms control agreements, legal export restrictions on dual-use goods and technologies, and possible other legal regulations, additional governance measures are needed to reduce the risk of misuse of bioscience research in general and synthetic biology in particular. All those working with biologically active substances should develop a strong awareness of safety and have knowledge of whom, if anyone, they can involve in assessing the danger of their projects without feeling unduly monitored. As may be the case in the U.S., where the Federal Bureau of Investigation [FBI] seeks to ensure preventive control of biosecurity threats and has systematically designated liaison officers for the DIY bioscene, among others).

In Germany, the dual-use problem with regard to biosecurity-relevant research projects ("Dual Use Research of Concern" /DURC) has been taken up with commitment and intensively discussed by scientific organizations, non-governmental organizations (NGOs) and politicians in recent years. As a result, the German government commissioned the German Ethics Council to prepare a statement on the topic of "Biosafety - Freedom and Responsibility in Science". This was presented in May 2014 and is likely to form the reference point for further political treatment of the topic in Germany in the coming years. The German Ethics Council calls for a legal regulation of dual use research of concern. Core points of the further recommendations are the creation of a nationwide, i.e. for all types of public and private research institutions, valid research code for a responsible handling of biosecurity issues as well as the establishment of a central, interdisciplinary DURC commission, which all researchers have to inform before conducting DURC projects.

With a view to the concrete reduction of abuse potentials of a significantly more powerful, cheaper and possibly decentralized gene (om)synthesis in the future, a reporting obligation for "gene synthesizing" facilities as well as a registration of DNA synthesizers also appears to be an option that could at least be tested - even if biocrime and bioterror risks are most likely to result from actors from organizations and countries that precisely cannot be controlled by (supra)governmental regulations.

3. Sustainable Models for the Protection and Use of Intellectual Property

The question of how intellectual property generated by modern life sciences can and should be protected is one of the most hotly contested in the genetic engineering debate, for both economic and ethical reasons. Among other things, with regard to future, for example "designed" molecular structures,



2019-1-BG01-KA203-062371

genes or even organisms, it should be noted that commercial protection will be much more plausible for these than for primarily analytical results in the form of naturally occurring DNA sequences. Another novelty is that, in addition to the established (bio)patent law, copyright law is increasingly being discussed as a future protection and utilisation concept. This applies in particular to the assumption that the future of Synbio will involve the design of biological information, including DNA, and then other molecules or properties of synthetic systems, similar to the programming of software codes.

For research policy, the question arises as to whether or which forms and projects of funding can be linked to specifications for access and conditions of use of the results. This question has been intensively discussed for years in science and politics far beyond the field of the life sciences. It is evident that the handling of intellectual property under the conditions of an increasingly digital economy will remain one of the major issues for science as well as research and economic policy in the coming years. The development of scientifically, economically, socially, politically and legally realistic, innovative regulatory models would be a very challenging, costly task for an in-depth technology assessment. The issue of intellectual property rights (IPR) is regularly associated with patents, although the latter are only one type of IPR, albeit the most important. A first question with regard to possible challenges in patenting synthetic biology inventions is whether the patent procedure for synthetic biology is significantly different from the current patenting system, and secondly, whether the traditional patenting system is effective enough to deal with the new developments. With regard to current patent law in general, we can state that the patentability of microorganisms and higher life forms, including genetically modified organisms, has been confirmed by the European Patent Convention and its case law. It is therefore not a problem specific to synthetic biology ("essence of life" issue).

4. Challenges of the foreseeable new genetic engineering debate

While the perspectives and potentials of Synbio in the restricted sense, i.e. the production of cells or organisms designed and constructed in a novel way "on the drawing board", still have the status of a vision of the future in spring 2015, the situation with synbio in the broader sense, understood as the next stage of biotechnology or genetic engineering, has changed massively in recent times. The discussion about the new possibilities and consequences of genome editing procedures has become so widespread and intensified in the last few weeks of the report's preparation that a fundamental change in the debate about the further development and use of gene manipulation techniques can be assumed.

It is foreseeable that the problem of a safety assessment or risk assessment without a substantially similar, familiar comparative organism will take on a much greater urgency if genome editing techniques are used worldwide in the coming period for the extensive modification of genomes. In this respect, an intensification of biosafety research is likely to be inevitable, both nationally and through international cooperation. The global extent and consequences of this development can hardly be predicted in detail. But it is clear that in the coming years, not only for research policy and many new, sometimes only renewed questions will arise about funding, socio-economic and ethical evaluation as well as regulation



2019-1-BG01-KA203-062371

of the applications of genetic engineering and bioethics, for which it ultimately does not matter much whether the technologies and processes are called synthetic biology. What is also new about this is the increased importance of the international dimension of the issues, which results not least from the growing and further increasing scientific and technological capacity of the emerging countries. Continuous monitoring of global developments using scientifically valid indicators and regular reporting therefore seem obvious.

5. Overall assessment

Overall, it can be concluded that the development and application status of synbio is not yet very advanced and that the future superiority and economic feasibility of synbio approaches cannot be seriously assessed. The latter applies in particular to the potential uses of synbio in the broader sense, which are still primarily visionary today. It is not foreseeable whether (more or less completely) artificial organisms or "bio-like" systems will ever become of major importance for efficient, reliable and safe "bio-based" production.

Synbio methods and processes in the broad sense must also assert themselves against existing options and others that are also being developed. Individual projects and products are already competitive today, mostly small-volume but high-priced products (speciality chemicals, flavourings, pharmaceuticals, vaccines). For these, neither cost issues nor biosafety aspects play such a major role because the existing or alternative processes are also costly and because either work can be done in safer closed systems (bioreactors) or potential risks/side effects are more readily accepted (pharmaceuticals/therapeutics). It should not be overlooked that the most discussed product examples of Synbio, the malaria drug artemisinin, the flavouring vanillin produced with the help of modified yeast cells and a palm oil substitute from microalgae, are not very far away from "conventional" genetic engineering applications.

The prospects of success of therapeutics, vaccines and gene therapy approaches cannot be assessed in general terms. In medicine in particular, efficacy and relative excellence often only become apparent in very late stages of development or even application. Therefore, the main benefit-risk debate on Synbio applications in the health sector is currently directed at other levels: at the ecological risks of using modified mosquito populations and at questions of global social justice in new methods of producing drugs and vaccines. The significance of Synbio is likely to vary greatly in the different areas of application depending on economic success and social acceptance, analogous to the situation with "conventional" (green, red and white) genetic engineering. The consumer-sensitive area of flavourings and fragrances or other ingredients for the food, cosmetics and detergent industries will occupy a special position.



CLONING OF ANIMALS AND HUMANS

1. Introduction

In 1997, the cloned sheep Dolly was presented to the world public. Since then, the topic of cloning has repeatedly made the headlines. She has three mothers and no biological father. She is genetically identical to one of her mothers. She is the first cloned mammal that is not the result of a new combination of father and mother, but was conceived from a body cell of one of her mothers. But while in Dolly's day some researchers vehemently opposed the cloning of human cells, today they themselves work with embryonic stem cells in the hope of someday combating diseases such as cancer or Parkinson's disease. Cloning, then, in the context of medicine, biotechnology and molecular biology, is the production of entities, individuals and populations that are genetically identical or nearly identical to the original organism or part of an organism from which they are derived. In its spontaneously occurring form, cloning is the way bacteria and some plants and animals reproduce asexually.

The most dramatically controversial area is human cloning for reproductive purposes, i.e. to produce babies that will grow into full-grown adults and full members of their society. Research on human embryos, including cloning with nuclear transfer, is widely permitted fourteen days after conception; and the subsequent cultivation and scientific and therapeutic use of human embryonic stem cells is accepted in most countries (not all). Human reproduction is at the heart of the cloning issue, ethically, with the ideas of design and the historically ever-popular theme of improving individuals and improving the human race.

Artificial cloning exploits the potential of particular, undifferentiated cells to differentiate into cells of a particular type under appropriate conditions. These cells are called stem cells. They are found both in small numbers in the body of an adult, to replace missing or dead cells there, and in early embryonic stages, from about the fourth to the seventh day after fertilisation. Only the embryonic stem cells up to about the eight-cell stage can still develop into all tissue types and thus into a whole organism; they are totipotent (= omnipotent). In contrast, no whole organism can be formed from all other stem cells. They can only give rise to many different cell types or only one specific cell type, they are pluripotent or multipotent.

2. Biomedical research and application

Clones of higher organisms are of great interest for biomedical basic research as well as application-oriented medical research. Currently, four main possible fields of application of nuclear transfer-based cloning for medical purposes are being discussed. A first area is so-called gene pharming, i.e. the use of transgenic animals to produce therapeutically useful (human) proteins, e.g. in milk. In the



2019-1-BG01-KA203-062371

foreseeable future, this will be one of the main potential applications of nuclear transfer-based cloning, as it makes the production of the corresponding transgenic animals more effective and targeted compared to conventional methods. Advantages of these active substances obtained by biogenetic manufacturing processes, such as insulin or blood factors or other human endogenous substances, are that these active substances can be obtained in a much purer way than in the conventional method via animal and human intermediates. If such animals are available, active substances can be produced in large quantities and relatively cheaply. However, there are also risks for the animals due to the genetic (transgenic) manipulation, the biological activity of the protein produced and the cloning process itself. Risks for humans can arise from changes in the products as well as from possible disease (pathogen) transmission, so they must be ruled out as far as possible by careful drug testing.

Another area where cloning could potentially be used is in the production of transgenic animals as animal models for human diseases. A major obstacle in the further development of animal models has been shown to be the fact that so far it has only been possible in mice to integrate genetically manipulated cells into the germ line of a recipient animal in such a stable way that the genetic changes can be inherited. However, the physiological and anatomical differences between mice and humans are so great that the symptoms of the genetic modification introduced in mice often do not correspond to the clinical picture observed in humans. Cloning by means of nuclear transfer using somatic cells opens up the possibility of inducing targeted genetic changes in different species (gene targeting and gene knockout). This would also make it possible for the first time to create disease models in transgenic large animals which, depending on the disease to be investigated, could be superior to previous mouse models in terms of anatomical, physiological or genetic characteristics. It is generally expected that in the medium term this will contribute to a better understanding of the clinical pictures of genetically caused human diseases and, based on this, to the development of effective treatment options. Cloning could also make a technical contribution to the transplantation of autologous tissue and to so-called cell therapy. The optimal transplant tissue is easy to characterise: its cells should be as genetically identical as possible to those of the recipient. The patient's immune system would then no longer recognise it as foreign, and any problem of rejection would be eliminated. Therefore, an optimal solution would be to create genetically identical replacement tissue. Research results suggest that this could now be achieved by means of nuclear transfer-based cloning. In principle, another way of cultivating human replacement tissue is conceivable: With the help of the nuclear transfer method, an early embryo would be created, from which pluripotent embryonic stem cells could be obtained in culture. However, it has not yet been possible to obtain such cells in humans, even from embryos created in vitro. Moreover, such a procedure would require the ethically and legally highly problematic creation and utilisation of a human embryo, unless oocytes from animals could be used as recipients of the cell nuclei. But this development is still in its infancy and involves problems of its own, especially ethical problems that are also serious.

A fourth area in which the use of (transgenic) cloned animals is conceivable is xenotransplantation (transplantation of animal organs into humans). However, in order to construct "donor animals", up to about a dozen genes would have to be altered in pigs, for example. This is practically impossible with conventional methods of genetic modification. Cloning could now make it possible to first provide cells in culture with the desired genetic changes before a multiply genetically



2019-1-BG01-KA203-062371

modified animal could be created from them with the help of nuclear transfer-based cloning. But even if the "ideal" donor animal could be created in this way, the fundamental problems of rejection would probably remain. It is also uncertain whether the foreign animal organ will actually fulfil its function in the human recipient. The problem of animal viruses adapting to humans also remains, with the possible consequence of epidemics.

3. Legal aspects

From a legal point of view, it is particularly important to answer the question of which regulations govern animal cloning in Germany (and abroad), and under which conditions cloning is or is not legally permissible. There is no explicit consideration of cloning techniques in the Federal Republic in the Animal Protection Act, for example. However, the cloning of animals could be covered by the provisions of Section 7 (1) of the Animal Protection Act, as this paragraph contains provisions on animal experiments and the cloning procedures are predominantly still at the experimental stage. However, the application and impact of this paragraph are discussed in very different ways: If one does not regard the de-nucleation of the egg cell as a genetic modification in the legal sense, the transfer of the egg cell into the gestating animal does not constitute an animal experiment either. However, if one comes to the conclusion that cloning by means of nuclear transfer falls under the provisions of Section 7, Paragraph 1, Sentence 2 of the Animal Protection Act, because this involves interventions on the genetic material and, in addition, the cloning experiments can be associated with pain or harm for the genetically modified animals (or carrier animals), cloning experiments by means of nuclear transfer would clearly be subject to authorisation.

From a constitutional point of view, a cloning ban in the Federal Republic of Germany would violate the fundamental rights of researchers and professionals under Article 5(3) (freedom of research) and Article 12(1) GG (freedom of occupation). A cloning ban or other restrictions on cloning would also constitute an encroachment on the constitutionally guaranteed freedom of science. A constitutional barrier that could justify the encroachment obviously does not exist. According to Article 12 (1) of the Basic Law, a ban on cloning, for example, would therefore be unconstitutional, as it would not be compatible with the public good and would not be covered by the legal reservation of Article 12 (1) sentence 2 of the Basic Law. The cloning of animals is thus permissible in principle under the current conditions and is subject to only limited restrictions under valid law. A "state goal of animal protection" does not exclude the use of animals by humans per se, but it increases the requirements for the necessary justification.



2019-1-BG01-KA203-062371

4. Ethical aspects

Different positions in the social discussion and evaluation of animal cloning can partly be traced back to different fundamental value assumptions. These also determine whether the cloning of animals is considered to have a new quality compared to conventional or other new methods of animal breeding. Some theologically based positions regard cloning, for example, as an intervention in creation to which humans have no right. Those who ascribe an "intrinsic value" or a "dignity of creation" to animals will generally consider animal cloning to be at least morally problematic. From an anthropocentric perspective, the question of the safety of products produced with the help of the cloning procedure and the ecological (impoverishment of genetic diversity) and social (industrial mass production, concentration of capital, new dependency relationships) risks and dangers possibly associated with its use are in the foreground. In view of the difficulty of reaching a moral consensus, it is necessary to consider which ethical principles should guide the possible use of animal cloning.

As a rule, ethicists consider goals in biomedical research and application to be of high priority if they are particularly urgent or even vital with regard to human health and can only be achieved with the help of cloning from higher animals. Objectives in the field of basic research can also be considered of high priority and justify cloning of higher animals if no alternative methods are available. However, should cloning be associated with considerable suffering for the animal concerned, it must be examined whether the mere interest of humans in knowledge already constitutes a sufficient reason for justification or whether justifications are only possible for certain objectives, i.e. when they are necessary to avoid considerable human suffering. Goals in the field of livestock breeding are usually mentioned as subordinate to the goals mentioned, unless they explicitly serve to secure the food basis for humans.

5. Conclusions and options for action

In applied research, nuclear transfer-based cloning opens up new ways to produce transgenic animals. Some therapeutically effective proteins can be produced cheaply in this way. The production of autologous replacement tissue appears to be promising from a medical and ethical point of view, and corresponding research activities are therefore particularly worthy of support. It is unclear whether it will be possible to create better examination models for human diseases in farm animals, but because of the not insignificant medical importance, efforts should also be intensified and supported in this area. Overall, the potential benefit of nuclear transfer-based cloning for the fields of research and medicine appears to be relatively high.

From an ethical point of view, an evaluation of animal cloning must in principle be based on the same criteria that are (or should be) applied to traditional animal breeding. In this regard, the establishment of a national ethics commission, which would have to deal with the moral-ethical questions of the progress of biological and biomedical technology as a whole or with the consequences



of progress in biology and medicine in the non-human sphere, is also problematised in various places. Its task would be to advise political decision-makers and inform the public.

Law and Ethics in the Field of Environmental Sustainability

INTRODUCTION

The principle of sustainability or sustainable development is the subject of a wide range of international, national and local activities, theoretical efforts, legal and planning measures. They are accompanied by an almost unmanageable abundance of publications and documentation. However, essential questions regarding the interpretation of this principle remain unanswered.

The principle of sustainability is widely understood on the basis of the 1987 report of the World Commission on Environment and Development (the so-called Brundtland Report), whose definition is often regarded as the standard: "Humanity has the ability to make development sustainable - to ensure that it meets the needs of the present without compromising the ability of future generations to meet their own needs. The core element of this conceptualisation is environmental protection from the perspective of intergenerational and international justice. However, the report contains a second, less well-known definition, which emphasises the radical social changes required and the process character of sustainable development: "Sustainable development is (...) a process of change in which the exploitation of resources, the direction of investments, the orientation of technological development, and institutional change are made consistent with future as well as present needs.

EUROPE'S COMMITMENT TO SUSTAINABLE DEVELOPMENT

Sustainable development has been at the heart of European policy for many years and the Treaties of Europe recognize the economic, social and environmental dimensions. Economic prosperity, efficiency, peaceful societies, social inclusion and responsibility, with dignity for all in their environment, is the basis of sustainable development. Sustainable development is therefore a cross-



2019-1-BG01-KA203-062371

cutting issue that affects all states. Europe is therefore obliged to meet the needs of the present and it must not risk that future generations will not be able to meet their own needs.

Ensuring sustainability is a challenge for Europe, because it ranges from youth unemployment, climate change, pollution, energy and migration policies to population aging. We must prepare for current and future challenges and respond to rapid and complex global changes and the needs of the world's growing population. To preserve the European social model and social cohesion, it is essential to invest in our youth, promote inclusive and sustainable growth, address inequalities and manage migration prudently. The sustainability of our health and pension systems will be improved by pursuing responsible fiscal policies and reforms, because if we are to protect our natural capital, we must accelerate the transition to a competitive low-carbon, climate-resilient and resource-efficient circular economy. Thus, a strong commitment to research and innovation is needed to turn these challenges into opportunities for new businesses and jobs.

REGULATION OF ENVIRONMENTAL BEHAVIOUR

The understanding of the sustainability principle is spread as a standard by the report of the World Commission on Environment and Development published in 1987: "Humanity has the ability to make development sustainable - to ensure that it meets the needs of the present without compromising the ability of future generations to meet their own needs".

Intergenerational and international equity makes environmental protection a core element of the conceptualization. But the report contains a second, less known definition, which emphasizes the necessary radical social changes and the process character of sustainable development: "Sustainable development is ((...)) a process of change in which the exploitation of resources, the direction of investments, the orientation of technological development, and institutional change are made consistent with future as well as present needs".

The so-called three-pillar model is, however, the definition most frequently used in the sustainability discourse: "Sustainability is the conception of a permanently sustainable development of the economic, ecological and social dimensions of human existence. These three pillars of sustainability interact with each other and require balanced coordination in the long term".

Sustainability is future-oriented and at the same time utopian, i.e. it is a utopia, but not in the sense of "illusory carelessness" but rather "as an expression of a departure into a future offensively oriented towards gaining new perspectives" - especially in view of the narrowing of future options by economic, ecological and social problems.

The EU is therefore committed to a development that meets the needs of the present without risking that future generations will not be able to meet their own needs. A life in dignity for all with the



2019-1-BG01-KA203-062371

resources available on this planet, characterized by economic prosperity, efficiency, peaceful societies, social inclusion and environmental responsibility, is the basis of sustainable development.

INSTRUMENTS

1. Instruments to enforce the environmental policy (environmental planning)

Environment policy has developed in the industrialised countries primarily as a reaction to a high environment intensive growth of the industry at the beginning of the seventies in the last century as a special government department. At first it confined itself mainly to the activity of the state. In the meantime however more and more environment relevant protagonists (so called “stakeholders”) are called to account for environmental matters. Especially the direct responsibility of the producer of (potential) environmental problems plays an increasing significant role. There is also the need to exert eco-political goals and strategies in other departments: e.g. in the energy -, transport -, and industry -, agricultural – or building and construction policy. “Hard” eco-political instruments (as laws and regulations) are side by side with the “soft” methods of behavioural control.

Beside the environmental law the environmental planning forms the central set of tools, insofar ecological policy wants to take effect not only as regulatory but also as formative policy. The environmental planning can be regarded as the development of sustainable environmental strategies, which are to facilitate the achievement of regional and/or sectoral environment protection goals within a certain timeframe: e.g. the reduction of CO₂-emissions by 25% within the next ten years. In the eighties of the last century the passage of national environmental plans in Denmark, Netherlands and Finland played a pioneering role in this. Therefore we will first expand on the possibilities of the environmental planning.

For enforcing the environmental policy principles and objectives two instruments are implemented in the legal framework of many states within the EU, means the different types of environmental planning and the different measures for regulation environmental behaviour. Environmental planning is an important means of precautionary protection. Such planning takes place as a multi-stage process, involving registering the current situation, forecasting future developments, and conflicts of objectives and interests. Plans can take the form of laws, statutory regulations, statutes, administrative regulations, or administrative acts, each of which has different legal consequences

Two forms of environmental planning are dominant: The so called “comprehensive planning”. It is the task of comprehensive planning concerning the environment is to determine, while exercising foresight, land use for residential, economic, and leisure purposes for a certain area, irrespective of any specific project and not limited to any specific sector. And the second one is sectoral planning: By contrast, sectoral planning is concerning the environment serves to establish environmental protection



2019-1-BG01-KA203-062371

plans. They are mainly the landscape plans, clean air plans, noise abatement plans, water conservation plans, and waste management plans, all of which require additional enforcement measures.

Another important instrument to enforce the environmental policy demands is the „Environmental impact assessment” (EIA). The primary objective of this instrument is to inform the administration in good time and comprehensively about the environmental impacts of environmentally significant projects. Environmental impact Assessment is to identify, describe, and assess all of the direct and indirect impacts of a planned project on the environment, including ecological interactions, in good time, thus allowing the taking of precautionary measures, across all media and sectors, and involving the public.

2. Instruments to regulate the environmental behaviour

Environmental behaviour is perhaps the most important objective for environmental policy and education. There are some instruments for regulation the environmental behaviour. One has to distinguish between direct and indirect forms of regulation:

Direct regulation of behaviour pertains to legal measures designed to immediately affect environmental behaviour. The “classical” instrument of this type is environmental regulatory law, which originates from police and regulatory law and generally punishing non-compliance by imposing sanctions. Accordingly, actions with adverse environmental impact are subject to administrative control, which is characterized by legal requirements of notification, registration, licensing, authorization, approval, and other procedures of granting permission to engage in such activity. In addition, direct regulation is also exercised by means of expressly (absolutely) prohibiting or requiring certain behaviour by law.

3. Principles for political and legal measures

Important is to distinguish and explain the principles guiding environmental law both in a national and an international frame and understand the meaning of ‘environmental sustainability’ and ‘sustainable development’ in the context of nature protection. Serious and substantial environmental law has to be guided by some high-ranking principles. For many international and national regulations in the field of environmental law within the European Union (e.g. in Germany), four basic principles are the basis for all processes of environmental law-making regarding the precautionary principle, the polluter-pays principle, the principle of sustainable development (concerning the integration of environmental protection and economic development) and the cooperation principle.

Other principles are often mentioned, which complete the four main principles or define them in a particular way. Some examples are Environmental procedural rights, common but differentiated



2019-1-BG01-KA203-062371

responsibilities, international and intergenerational equity, common concern of humankind and the common heritage.

PRECAUTIONARY PRINCIPLE

In its origin, the precautionary principle is rather a political than a philosophical principle and was first introduced as ‘Vorsorgeprinzip’ (principle of precaution) in the German-speaking area. It was incorporated into several national legal texts and international treaties or declarations. A good definition was given by Per Sandin: “The basic message of the precautionary principle is that on some occasions, measures against a possible hazard should be taken even if the available evidence does not suffice to treat the existence of that hazard as a scientific fact.” It can therefore be stated that the precautionary principle is based on hazard detection and scientific uncertainty. As a consequence, the burden of proof (that an action might cause severe harm to the public or the environment) falls on those who plead for measures to prevent such an harm. Whenever one can anticipate plausible harm for society or the environment, the precautionary principle should be applied. But often it is not clear whether a planned action will cause harm to the public or the environment or not, because the possible impact of human actions on the environment or human health often depends on the dynamics of complex systems, so the real consequences of actions may be unpredictable. Therefore further scientific research is required – but also caution if a current action intervenes in complex (human or natural) systems.

Nowadays the precautionary principle is incorporated into many European and international contracts and treaties. In its 1976 Report on the Environment, for example, the German Federal Government describes the precautionary principle as follows: Environmental policy is not limited to averting imminent danger and remedying damage that has already occurred. Precautionary environmental policy furthermore demands that the natural environment be protected and treated with care. The precautionary principle is embodied in a number of environmental provisions, and also involves resource conservation in addition to risk precaution.

The precautionary principle is especially important in legal regulations and decisions concerning potential risks to public health, such as the marketing of genetically modified foods, the use of growth hormones in cattle raising, or measures to prevent ‘mad cow’ disease.

Nevertheless, in real cases the policy-makers often have to struggle with a lack of valid scientific information or with irreducible conflicts between the interests of different stakeholders. Sometimes it is very difficult to estimate or assess the potential harm and to find an acceptable political compromise. But anyway, rigorous application of the precautionary principle should be avoided when there is insufficient knowledge of whether there is a real potential risk from an innovative product or an activity or not. In this case the principle could be taken immoderately as an absolute ban on all actions - which could stall all technological innovation and progress.



POLLUTER-PAYS PRINCIPLE (VERSUS COMMUNITY-PAYS PRINCIPLE)

The polluter-pays principle states that the one causing environmental impact is principally held responsible - materially and financially - for protecting the environment and is required to prevent, correct, or financially compensate such impact. But a problem arises in cases of inherited pollution where the responsible parties often cannot be held liable and - if no other party can be held responsible - the general public must bear the cost. In such cases the polluter-pays principle would be replaced by the community-pays principle.

In environmental law, the polluter-pays principle is enacted to make the party responsible for producing pollution responsible for paying for the damage done to the natural environment. It is regarded as a general custom because of the strong support it has received in most Organisation for Economic Co-operation and Development (OECD) and European Community (EC) countries. In international environmental law it is mentioned in Principle 16 of the Rio Declaration on Environment and Development (1992).

The polluter-pays principle is an important element of environmental policy and influences, for example, political measures for reducing greenhouse gas emissions. Often this principle will be applied as the so-called 'extended polluter responsibility' (EPR). This concept was probably first formulated by the Swedish government in 1975. For instance, EPR can help to shift the responsibility for dealing with waste from governments and taxpayers to the real producers of the waste. OECD defines EPR as: a concept where manufacturers and importers of products should bear a significant degree of responsibility for the environmental impacts of their products throughout the product life-cycle, including upstream impacts inherent in the selection of materials for the products, impacts from manufacturers' production process itself, and downstream impacts from the use and disposal of the products. Producers accept their responsibility when designing their products to minimise life-cycle environmental impacts, and when accepting legal, physical or socio-economic responsibility for environmental impacts that cannot be eliminated by design.

THE PRINCIPLE OF SUSTAINABILITY (SUSTAINABLE DEVELOPMENT)

Another important principle is the principle of sustainable development, which may be viewed as an instance of applying the precautionary principle to resources. This principle is a pattern of resource use that aims to meet human needs while preserving the environment so that these needs can be met not only in the present, but also for future generations. For the first time, the term "sustainable development" was used by the Brundtland Commission (1987), which has given the most famous definition of sustainable development as development that "meets the needs of the present without compromising the ability of future generations to meet their own needs" (United Nations 1987).

The term 'sustainable development' seeks to combine the resources and processes of natural systems with the human needs and economic activities of social systems. Already in the 1970s the term



2019-1-BG01-KA203-062371

‘sustainability’ had been used for an economy “in equilibrium with basic ecological support systems”. On the base of the idea of sustainability and according to the alarming theses of “The Limits to Growth” many ecologists tried to create the new concept of a “steady state economy”, especially with respect to environmental concerns. In this context, ‘sustainable development’ does not refer solely to environmental issues, but also takes into account social and economic considerations: the resolving of conflicts between different competing goals and stakeholders, and the harmonising of economic growth and social welfare with environmental quality. The concept of sustainable development—both of nature and society – points out that the survival of mankind depends essentially on the survival of nature (or the natural environment), because economic and socio-cultural welfare is directly coupled with the the welfare of nature – resources, plants, animals, etc. Ultimately, the exploitation and degradation of nature can result in the inability to maintain human life and even in the extinction of mankind. The theory of sustainable development is therefore based on the assumption that societies have to manage three forms of non-substitutable capital: economic, social and natural capital.

It may be that we can find ways to replace some natural resources, but it is unlikely that we will ever be able to replace the services provided by the eco-system: for example, to protect us against dangerous cosmic radiation with an intact ozone layer, or to supply us with sufficient oxygen as the tropical forests or the algae of the oceans do. The multi-functionality of many natural resources and also biodiversity are irreplaceable. Moreover, the deterioration of natural resources and the loss of natural services (e.g. the absorption of nutrients by a lake) are often irreversible processes – like the loss of ethnic and cultural diversity (e.g. indigenous languages). Therefore only a sustainable development can secure both: the protection of a functional intact environment and the survival and welfare of human beings.

COOPERATION PRINCIPLE

‘The cooperation principle underscores that environmental protection is the responsibility of all of society and not just of the state: accordingly, all parts of society and the state are called on to cooperate’ (Knopp 2008: 49) The cooperation principle is the weakest of the four environmental principles, and it can hardly be considered as satisfying the requirements demanded of a guiding principle of law.

OTHER PRINCIPLES IN NATIONAL AND INTERNATIONAL ENVIRONMENTAL LAW

Apart from the four basic principles, there are a number of others guiding national and international environmental law, such as the ‘grandfathering principle’ or the ‘principle that action may not result in a significant deterioration of environmental conditions’. Last but not least, we should also mention the principle of trans-boundary environmental protection: this principle mirrors the insight that



2019-1-BG01-KA203-062371

environmental problems do not stop at national borders. For instance, this principle underpins much of the Water Framework Directive of the European Union where it covers the trans-boundary management of water resources in natural river basins.

National as well as international environmental laws are often based on the above called principles, especially the trans-boundary principle. This is important, because many environmental problems are border-crossing problems, for example, climate change, sea water and air pollution.

REGULATION OF ENVIRONMENTAL BEHAVIOUR

1. Instruments to enforce environmental policy (planning)

Environmental policy has developed in the industrialised countries primarily as a reaction to the highly intensive growth of the environment industry at the beginning of the 1970s into special government departments. At first policy confined itself mainly to the activity of the state. Over the years, however, more and more protagonists with any interests in the environment field (so-called 'stakeholders') are being called to account on environmental matters. In particular, the responsibility of the producer of (potential) environmental problems is becoming increasingly significant. There is also a need to exert eco-political goals and strategies in other departments, for example, in policy for energy, transport and industry, agriculture, and building and construction. 'Hard' eco-political instruments (such as laws and regulations) exist side by side with the 'soft' methods of behavioural control (such as education of engineers concerning environmental awareness), for example, in the case of projects that involve many private stakeholders or the public.

Besides environmental law, environmental planning forms are a central set of tools to the extent that environmental policy tries to operate not only as a regulatory but also as a formative instrument. Environmental planning can be regarded as the development of sustainable environmental strategies to facilitate the achievement of regional or sectoral environment protection goals within a certain time-frame, for example, the reduction of CO₂ emissions by 25% within the next ten years. In the 1980s the implementation of national environmental plans in Denmark, Netherlands and Finland played a pioneering role in this. We will, therefore, first expand on the possibilities of environmental planning.

To enforce environmental policy principles and objectives two instruments are implemented in the legal framework of many states within the EU that means different types of environmental planning and the different measures for regulating environmental behaviour.

Environmental planning provides an important means of precautionary protection. Planning takes place as a multi-stage process, involving registering the current situation and forecasting future developments; moreover, it has to take into account possible conflicts of interests.



2019-1-BG01-KA203-062371

Plans can take the form of laws, statutory regulations, statutes, administrative regulations or administrative acts, each of which has different legal consequences. In addition, environmental planning may involve comprehensive planning or sectoral planning. Two forms of environmental planning are dominant, the comprehensive planning. The task of comprehensive planning is 'to determine, while exercising foresight, land use for residential, economic and leisure purposes for a certain area, irrespective of any specific project and not limited to any specific sector' and the sectoral planning. By contrast, sectoral planning serves to establish environmental protection plans for specific sectors, chiefly landscape, clean air, noise abatement, water conservation and waste management, all of which require additional enforcement measures

Another important instrument for enforcing environmental policy demands is environmental impact assessment (EIA). The primary objective of this instrument is 'to inform the administration comprehensively and in good time about the environmental impacts of environmentally significant projects' EIA is used to identify, describe and assess all of the direct and indirect impacts of a planned project on the environment, including ecological interactions, in good time, thus allowing precautionary measures to be taken across all media and sectors, and involving the public.

2. Instruments to regulate environmental behavior

Environmental behaviour is perhaps the most important target for environmental policy and education. There are various instruments for regulating environmental behaviour, which can be distinguished as direct or indirect forms of regulation: as (1) direct regulation and (2) indirect regulation of behavior.

DIRECT REGULATION OF BEHAVIOUR

Direct regulation of behavior pertains to legal measures designed to immediately affect environmental behaviour. The traditional instrument of this type is environmental regulatory law, 'which originates from police and regulatory law and generally punishes non-compliance by imposing sanctions'. Accordingly, actions with adverse environmental impact are subject to administrative control, which is characterised by legal requirements of notification, registration, licensing, authorisation, approval and other procedures of granting permission to engage in such activity. In addition, direct regulation is also exercised by means of expressly prohibiting or requiring certain behaviour by law.

Absolute legal bans (e.g. in Germany under the Federal Nature Protection Act, 2002, §§ 23 [2], 42 [1] and [2]), directly forbid certain behaviour with adverse impact on the environment. However, legislators only rarely employ measures of this type. By contrast permission procedures are the key instrument in current environmental regulatory law in many European states. Projects subject to



2019-1-BG01-KA203-062371

permission are strictly prohibited without permission. ‘Erecting or operating an installation of environmental significance, using environmental media, or producing and distributing certain products may all be subject to permission’. Thus a permit is a constitutive administrative act in that it grants the applicant the right of lawfully engaging in an otherwise prohibited activity. Environmental law includes a number of so-called environmental obligations, of which basic obligations are of special significance. They impose certain obligations either on everyone or on a certain group of people. Normally, these basic obligations involve preventive and precautionary measures, most notably the conservation of resources (e.g. water or soil). Apart from those basic obligations, there are ‘numerous collateral obligations that may benefit the environment, such as promotion and performance obligations, monitoring and protection obligations, obligations to cooperate and continuously disclose information, organisational obligations and obligations to tolerate certain actions.

INDIRECT REGULATION OF BEHAVIOUR

Indirect regulation of behaviour does not rely on norms mandating behaviour, but aims to influence motivation: incentives are provided for environmentally friendly behaviour while leaving discretion to the addressee. The means of indirect regulation behaviour notably include informational instruments, economic instruments, such as levies certificates, and subsidies.

Information, appeals and warnings, means that according to the German Environmental Information Act (1994), providing free access to environmental information is viewed as a means of sharpening the awareness of citizens and public authorities of the need for effectively protecting the environment. These means of raising environmental awareness range from political and moral appeals to warnings, recommendations and other forms of information, such as labels and product and usage information. The most important means to indirectly regulate behaviour are environmental levies. ‘They place a price tag on the use of the environment and leave it to market participants to decide if and how they will react based on their individual cost–benefit analyses’. In practice, the inability to precisely affect behaviour via environmental levies can pose a problem. If they are set too low, polluters will opt for paying the levy instead of altering behaviour harmful to the environment. If levies are set too high, they may impede economic competitiveness. For instance, the following environmentally relevant charges are being levied in Germany in 2012 for example waste water charges, countervailing charges under nature conservation law and forest protection charges in various German States, water abstraction fees in some German States (‘water penny’) and the waste transportation charges (consumer law).

Environmental levies may be imposed as taxes, fees and contributions for benefits incurred, and special levies. Granting benefits to users of environmentally friendly products, means ‘Benefits for use’ refers to provisions that relax or lift general limitations imposed on the use of environmentally harmful products in the case of products that comply with standards that, although not required by law, are considered desirable, thus rendering such a product more environmentally friendly than others of the same kind. ‘Although this instrument does not involve financial incentives in the medium and long term,



2019-1-BG01-KA203-062371

changes in consumer behaviour may be expected that may lead to crowding environmentally more harmful products out of the market’.

Or the subsidies, that means providing financial assistance is a form of indirect behaviour regulation. Subsidies are monetary or non-monetary benefits granted by the state, without any product or service being provided in return. Subsidies are generally viewed with scepticism, since they are considered to be prone to abuse and to place the cost burden of environmental protection on the general public. In the European Union there has been a tendency to cut back on environmental protection subsidies.

And finally the idea of environmental certificates is based on a market-compatible form of quantity control by the state. Certificate-based schemes do not take prices as their starting point but define an admissible level for a certain future use of the environment in quantitative terms, leaving the formation of process up to the market. This instrument has been employed for climate protection under the Kyoto Protocol. The allocated emission allowances grant the holder the right to pollute the environment only to a certain extent. Should the holder pollute the environment to a lesser degree than permitted, the holder may sell the unused pollution allowances to another polluter. ‘Enterprises may thus elect to either reduce emissions from their installations or to acquire additional emission allowances from other enterprises that have been able to reduce emissions at lower cost’. Future experience will show whether this instrument will indeed prove successful in reducing greenhouse gas emissions. Economic instruments are gaining increasing significance as a complement to environmental regulatory law. There is no single answer to the question as to what is actually the ‘proper’ choice of instruments in order to achieve an adequate balance between various environmental user interests, the interests of affected neighbours, the interests of the general public and the protection of the environment. Legislators and administrations are thus ultimately compelled to rely on trial and error to reach an appropriate decision.

THE MULTIDIMENSIONAL SUSTAINABILITY STRATEGY

Social justice, prosperity and peace, with nature to overcome global crises, are to be seen as three interrelated and equally weighted goals of sustainability. But it remains unclear how to make a multidimensional sustainability strategy politically viable in individual countries as well as in the global community. There is indeed a great danger that this promising societal strategy will end up in the vicinity of utopia and wishful thinking based only on moral normative grounds.

An integrative sustainability strategy in the comprehensive sense is first of all about coordinating the different normatively based life perspectives of individuals, social groups, nations, present and future generations. In the process of searching for and shaping a globally sustainable development, innumerable agreements will have to be reached, both within society and internationally, which would have to be morally motivating for all actors involved. So motivating, in fact, that these agreements could attain a



2019-1-BG01-KA203-062371

degree of binding force that would allow possible violations of the agreements to be punished with sanctions. The consensus to be reached therefore goes beyond the mere coordination of different, normatively based perspectives: rather, it presupposes a generally accepted ethical framework as well as principles and standards that are ethically valid for all participants. In other words: Sustainability needs a morally suitable, politically viable and pluralistic guiding ethic that is socially and spatially and temporally transcendent, that has a high level of acceptance comparable to fundamental freedoms, and that allows operationalizable and targeted, detailed standards to be developed for ecological, economic, social, political and cultural sustainability dimensions. However, this ethic has been lacking up to now.

Precisely because of the lack of an acceptable ethics of sustainability, the uncertainty of formulating social and economic sustainability rules and justifying morally consensual action steps remains very high. Moreover, the lack of an acceptable and comprehensive ethics of sustainability favors the current dominance of one-dimensional, ecological-economic considerations in the sustainability debate and at the same time hinders the coordination, cooperation and mutual adaptation of promising actually integrative sustainability approaches and their respective goals.

The debate on ethics, which was originally conducted independently of the sustainability debate, has not yet provided any decisive impetus either, although the irreversible consequences of scientific and technological development have triggered a lively ethical debate on the responsibility of the present towards future generations. This debate was followed by the discussion on ecological justice and, more recently, on sustainability. Understandably, the first step in this discussion is to examine the extent to which previously accepted ethics of justice can be applied to ecological justice. The meager result of this discussion was, however, pre-programmed. The common justice ethics suffer from the one-dimensionality of their frame of reference. In them, social justice is a parameter of some other overarching goal.

- in utilitarianism, social justice is dependent on the maximization of total utility;
- in Marxism, social justice is only possible in a communist society, i.e., when the conditions for the equality of all people have been historically established;
- and in liberalism, social justice is a parameter of the goal of the greatest possible basic freedoms.

Seen in this light, these ethics are already inadequate for treating social justice as an independent and immediate socio-political goal. Their inadequacies become even greater, and their binding force for policy becomes weaker, if they were also to provide moral standards of value for additional and qualitatively new dimensions of justice, such as ecological, international, and intergenerational justice.



Equal opportunities as a universal ethic of globally integrative sustainability

The question thus arises whether a different ethics that takes into account the requirements of integrative sustainability is conceivable. In the opinion of the author and on the basis of his insights gained so far, which of course have a provisional character, equal opportunities as an independently conceived universalistic ethics could fill the demonstrably existing orientation gap. The core of his considerations is the definition of equal opportunities as "equal starting conditions for individuals, social groups, peoples of different color, religion, culture, language, for people of different genders, and for different generations to determine their own needs, lifestyles, and options, and to have equal access to natural resources, goods, and positions. Equality of opportunity is a condition that must be constantly re-established against both historically evolved and newly emerging inequality trends".

However, it needs to be argued in more detail whether and in what way equality of opportunity, understood in this way as a universal action-oriented ethic, can make a central contribution to overcoming the deficits outlined above for a policy of inclusive sustainability. The following considerations are taken as a starting point: The conclusion of the prevailing liberal view that the realization of equality of opportunity "precisely because of the principled universality of the individual reference cannot be specified in terms of content" and that "the magic and allure, the seductiveness and vagueness" make this concept "universally and all-roundly usable as a political fighting term" is logically not at all compelling. Every organized individualistic society must follow general norms and rules in the interest of all individuals. The universality of the idea of equality of opportunity consists precisely in the fact that individuals both animate each other to mutual claims and enter into obligations among themselves. It corresponds to the moral standards and conceptual logic of the principle that no individual may impair the opportunities of other individuals entirely in the sense of Kant's categorical imperative "act in such a way that the maxim of your will could at the same time at any time be regarded as the principle of a general legislation." Furthermore, the substantial as well as indispensable condition of equality of opportunity is equality of starting conditions. This condition is morally as well as logically integral to the principle.

The exclusion of historically grown inequalities, fortunes and positions, which have not arisen on one's own merit but by allocation, precludes the realization of equality of opportunity. In this respect, the assumption of vagueness and complete openness of the principle for political practice is an arbitrary one and results rather from the conception of justice of classical liberalism itself. Equality of opportunity can be interpreted not only intrageneratively, but also in an intergenerational sense universal interpretation. Kant's categorical imperative, strictly speaking, becomes normatively consequential only through an ethics of equal opportunity, thus overcoming its reputation of a merely formal principle with which no justifications of particular ends or maxims can be provided. With regard to its inter-generationally universal scope, the idea of equal opportunity also takes into account the legitimacy



2019-1-BG01-KA203-062371

dilemma often problematized by ethicists but not solved: Every society would have its own ideas of needs and well-being. The present generations would not have the right to define the needs of future generations and, moreover, to prescribe the technological and social conditions for them. This objection cannot be denied a comprehensible moral justification.

The positive turn of this objection, however, leads to the moral maxims of action for present generations that allow future generations equal opportunities to use nature according to their ideas of need, well-being, and happiness. "Our ignorance should not serve as a justification for limiting the life chances of those to come".

Weighty arguments thus condense and underpin the view that equality of opportunity can be made fruitful as a foundation for a social theory of integral sustainability that transcends space and time across disciplines. Equal opportunity as a universal ethic and integrative sustainability as a multidimensional framework for action require an inter- or transdisciplinary (socioeconomic, ecological, political science, sociological, and philosophical) approach.

The following principles, which are still preliminary are considered to be fundamental for specifying and achieving equal opportunities:

- Principle of freedom: Every human being has the same right to the most extensive total system of equal basic freedoms possible for all. A less extensive freedom must strengthen the overall system of freedoms for all (Rawls' first principle).
- Diversity principle: Every human being has the right to cultivate and maintain specific characteristics of his or her own, such as aptitude, lifestyle and life planning, and to use them in the sense of his or her own self-realization.
- Autonomy principle: Every person has the right to the fruits of his or her own labor (the idea of self-ownership according to classical liberalism and Marxism).
- Freedom of access principle: Every person has the same right of access to natural resources and to social positions. A restriction of this right must lead to the strengthening of the same for all people living in the present as well as for future generations.
- Principle of care: Everyone is obliged to care for disadvantaged and dependent people. The restriction of autonomy accepted in this process must strengthen the overall system of autonomy for all. The definition of equal opportunities and the formulation of its principles are preliminary. It remains to be verified to what extent both the definition of equal opportunity and its individual principles are complete, each individual principle is consistent in itself and these together can be integrated into an overall concept, and finally whether these individual principles can also be anthropogenically underpinned.

The question of the hierarchy of these principles must remain open for the time being; whether an evaluative hierarchy or equal ranking is morally compelling requires detailed investigation, although there is already much to suggest that these principles would have to stand in an indissoluble relationship to each other. However, there is sufficient evidence for the hypothesis that equality of opportunity meets



2019-1-BG01-KA203-062371

the requirements of a multidimensional ethics and the politics of integral sustainability much more strongly than the ethics of justice known so far. It is conceived as an integrative further development of those common ethics of justice in which either the principle of equality or the principle of freedom is absolutely dominant. Freedom, autonomy, self-realization and care, justice of achievement and justice of need give equality of opportunity a moral fitness and political capability of the highest order.

References

- Boldt et al. 2009. Synthetische Biologie - Eine ethisch-philosophische Analyse, p. 8.
- Catenhusen WM. 2011. Simultanmitschrift der Tagung des Deutschen Ethikrates vom 23.11.2011, p. 85.
- Charisius H., et al. 2012. Unser kleines Gen-Labor,
- Cohen J. 2012. WHO Group: H5N1 Papers Should Be Published in Full, Science February 24, Vol. 335 no. 6071, pp. 899-900 DOI: 10.1126/science.335.6071.899.
- Colussi IA. 2012. Synthetic biology, concerns and risks: looking for a (constitutionally oriented) regulatory framework and a system of governance for a new emerging technology, Trento.
- Dederer HG. 2010. Neuartige Technologien als Herausforderung an das Recht - dargestellt am Beispiel der Nanotechnologie, in: Spranger/Tade, Aktuelle Herausforderungen der Life Sciences, p. 71 f.
- Deutscher Ethikrat (German Ethics Council) (Friedrich, Bärbel 01/24/2010).
- DFG - Deutsche Forschungsgemeinschaft (German Research Foundation) (2009): Synthetic Biology, Bonn.
- Third Report of the Federal Government on Experience with the Genetic Engineering Act. .2008. Bt-Drs 16/8155, printed in: Eberbach et. al. (2012): Volume 2, Part I, B. I., p. 3.



2019-1-BG01-KA203-062371

- Eberbach W. 2012. Gentechnik und Recht, in: Eberbach et al., Recht der Gentechnik und Biomedizin, 79th Ergänzungs - Lieferung, Vol. 1, Part A. I. p. 13 (12).
- Engelhardt M. 2010. The Political Opinion, 493: 23.
- Fouchier, RA. 2012. Airborne transmission of influenza A/H5N1 virus between ferrets. Science, 336 (6088): 1534-41.
- Garfinkel MI, Endy D, Epstein GL, Friedmann RM. 2007. Synthetic genomics: options for governance. The J Craig Venter Institute, Rockville, Maryland, p. 38 ff.
- Jarass HD. 2013. Charter of Fundamental Rights of the European Union.
- Kluth W. 2012. Wissenschaftsfreiheit vs. Sicherheitsinteressen http://www.academics.de/wissenschaft/wissenschaftsfreiheit_vs_sicherheitsinteressen_52504.html.
- Krämer L. 2013. Genetically Modified Living Organisms and the Precautionary Principle, <http://www.testbiotech.org/node/904>
- Luttermann C. 2011. Synthetic Biology: Building Blocks for Life and Jurisprudence. JZ, 195.
- Mooney P. 2010. Next Bang! Wie das riskante Spiel mit Megatechnologien unsere Existenz bedroht, Munich.
- Nouri A, Chyba CF. 2009. Proliferation-resistant biotechnology: an approach to improve biological security. Nature Biotechnology, 27: 234 - 236.
- Parliamentary Ethics Committee of 07/01/2009, 16/13780.
- Presidential Commission for the Study of Bioethical Issues. 2010. New Directions. The Ethics of Synthetic Biology and Emerging Technologies, Washington, p. 140 ff.
- Robiński J, Simon J, Paslack R. 2016. Legal Aspects of Synthetic Biology. In: Joachim Boldt (Hg.): Synthetic Biology, Bd. 493. Wiesbaden, pp. 123–140
- Sauter A. 2011. Synthetische Biologie: Finale Technisierung des Lebens – oder Etikettenschwindel. TAB-Brief, 39: 23.
- Schmidt M. 2011. Biosicherheit und Synthetische Biologie. In: Pühler, A., Synthetische Biologie – Die Geburt einer Technikwissenschaft, p. 112 f.
- Schmidt M, Giersch G. 2011. DNA Synthesis and Security, In: Marissa J. Campbell, DNA Microarrays, Synthesis and Synthetic DNA, Chapter 6.
- Security and Defense Research - Working Group (2010): Guidelines and Rules of the Max Planck Society On A Responsible Approach To Freedom Of Research And Research Risks, 19. 3., www.mpg.de/232129/researchFreedomRisks.pdf - (accessed 11/17/2020).



2019-1-BG01-KA203-062371

Statement of NSABB. 2012. Meeting of the National Science Advisory Board for Biosecurity to Review Revised Manuscripts on Transmissibility of A/H5N1 Influenza Virus, oba.od.nih.gov/.../biosecurity/.../NSABB_Statem... (accessed 12/27/2020).

The European Group on Ethics in Science and New Technologies to the European Commission (cit. EGE). 2009. Ethics of synthetic biology, Opinion No. 25, Brussels, 17. November, p. 27 f.

Then C, Hamberger S. 2010. Synthetische Biologie, Teil 1: Synthetische Biologie und künstliches Leben – eine kritische Analyse, Testbiotech, June 2010.

World Health Organization, Statement (2011): WHO concerned that new H5N1 influenza research could undermine the 2011 Pandemic Influenza Preparedness Framework (11/30/2011), www.who.int/entity/.../news/.../index.html

ZKBS. 2012. Zwischenbericht der Zentralen Kommission für die Biologische Sicherheit. Monitoring der Synthetischen Biologie in Deutschland, p. 8.

Nida-Rümelin J (Hg.). 1996. Angewandte Ethik. Die Bereichsethiken und ihre theoretische Fundierung. Ein Handbuch, Stuttgart

Nida-Rümelin J, Von der Pfordten D, Tierethik II. 1996. Zu den ethischen Grundlagen des Deutschen Tierschutzgesetzes. in: Nida-Rümelin, pp. 484-509

Niemann H. 1997. Vermehrung genetisch identischer Tiere durch Klonen. Manuskript und Beantwortung des Fragenkataloges zur Anhörung im Ausschuss für Ernährung, Landwirtschaft und Forsten des Deutschen Bundestages am 11.6.1997.

Podschun TE. 1999. Sie nannten sie Dolly - Von Klonen, Genen und unserer Verantwortung, Weinheim.

Thomson JA, Marshall VS. 1998. Primate Embryonic Stem Cell Lines. Curr. Top. Dev. Biol., 38: 133-165

Tinneberg HR, Ottmar C. 1995. Moderne Fortpflanzungsmedizin - Grundlagen, IVF, ethische und juristische Aspekte, Stuttgart

Travis J. Human Embryonic Stem Cells Found?, in: ScienceNewsOnline,

Altner G. 1982. Grundlagen. In: Kalberlah, F., Michelsen, G. & Rühling, U. (eds), Der Fischer Öko-Almanach. Daten, Fakten, Trends der Umweltdiskussion, Frankfurt am Main, pp.13-50 (16).

Berkes F, Colding J, Folke C. 2003. Navigating social-ecological systems: building resilience for complexity and change, Cambridge University Press, Cambridge.

Bick H. 1987. Ökologie - Wissenschaft von den wechselseitigen Beziehungen zwischen Organismen und Umwelt. In: Calließ, J. & Lob, R.E. (eds), Handbuch Praxis der Umwelt- und Friedenerziehung. Vol. 1: Grundlagen, Düsseldorf, pp.16-27 (21).



2019-1-BG01-KA203-062371

Bückmann W, Leo YH, Simonis UE. 2003. Nachhaltigkeit und das Recht, Bundeszentrale für politische Bildung, 1.7.2003, Aus Politik und Zeitgeschichte (B27/2003), Umwelt und Klimapolitik

Bundesregierung. 2002. Perspektiven für Deutschland. Unsere Strategie für eine nachhaltige Entwicklung, Berlin.

Enquete-Kommission des Deutschen Bundestages. 1994. Schutz des Menschen und der Umwelt, Die Industriegesellschaft gestalten. Perspektiven für einen nachhaltigen Umgang mit Stoff- und Materialströmen, Bonn.

Meadows DH, Meadows DL, Randers J, Behrens III, William W. 1971. The Limits to Growth; A Report for the Club of Rome's Project on the Predicament of Mankind, New York.

Partelow S. 2018. A review of the social-ecological systems framework: applications, methods, modifications, and challenges. *Ecology and Society*, 23(4): 36.

Paslack R. 1991. Urgeschichte der Selbstorganisation. Zur Archäologie eines wissenschaftlichen Paradigmas. Vol. 32, in series: Wissenschaftstheorie: Wissenschaft und Philosophie. Braunschweig/Wiesbaden.

Paslack R. 2012. The challenge to environmental ethics, in: Vromans, K., Paslack, R., Isildar, G. Y., deVrind, R. & Simon, J. W. (eds), *Environmental Ethics – An Introduction and Learning Guide*. Greenleaf Publishing, Sheffield, pp. 65-82.

Sandin P, Peterson M, Hansson SO, Rudén C, Juthe A. 2002. Five charges against the precautionary principle. *Journal of Risk Research*, 5 (4): 287-299.

Stivers PE. 1976. The Debate Goes On: Science and Policy; Policy and Science, April 1.

UBA. 2002. Nachhaltiges Deutschland. Wege zu einer dauerhaft-umweltgerechten Entwicklung, Berlin 1997; auch in Englisch: *Sustainable Development in Germany. Progress and Prospects*, Berlin 1998; vgl. auch UBA, *Nachhaltige Entwicklung in Deutschland. Die Zukunft dauerhaft umweltgerecht gestalten*, Berlin.

Van den Belt H. 2003. Debating the Precautionary Principle: “Guilty until Proven Innocent” or “Innocent until Proven Guilty”? pp. 1122-1126.

Weidner H. 1995. 25 Years of Modern Environmental Policy in Germany. Treading a well-worn path to the Top of the International Field, Wissenschaftszentrum Berlin für Sozialforschung, pp. 1-99.



Project website: www.digit-biotech.eu

The European Commission's support for the production of this publication does not constitute an endorsement of the contents, which reflect the views only of the authors, and the Commission cannot be held responsible for any use which may be made of the information contained therein.