EMGEN Newsletter

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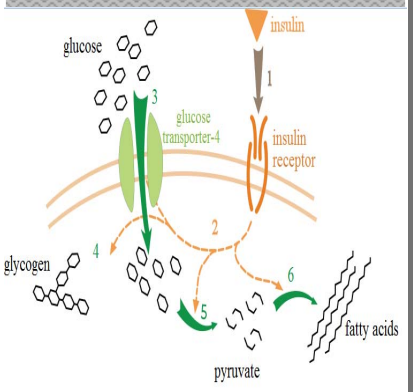

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CLONING

In natural science, the term cloning is a technique for generating identical beings with equivalent genotypes. The cloning take place when creatures like plants, bacteria or insects replicate no sexually. Cloning in modern technologies, such as biotechnology, discusses about procedures to generate duplicates of DNA segments (molecular cloning), cells (cell cloning), or organisms.

1. Natural cloning

Cloning is a natural procedure of reproduction that has permitted life to extend for more than 20 centuries. It is the reproduction process utilized by plants, bacteria, and fungi, and is similarly the approach that clonal colonies replicate themselves. Instances of these beings comprise, the Pando trees, the Kentucky coffee tree, hazel trees, Myricas, blueberry plants, and the American sweet gum.

2. Molecular cloning

Molecular cloning states the procedure of constructing several molecules. Cloning is normally utilized to intensify DNA fragments covering entire genes, but it can also be utilized to intensify any DNA sequence; for example, promoters, randomly selected DNA, and non-coding sequences. It is used in an extensive group of biological trials and practical applications from genetic fingerprinting to large scale protein manufacture. Sporadically, the word cloning is deceptively used to mention the recognition of the chromosomal location of a gene related to a specific phenotype of interest, for example in positional cloning. Actually, localization of the gene to a chromosome or genomic region does not essentially allow one to separate or intensify the relevant genomic sequence. To intensify any DNA sequence in a living organism, that sequence need to be connected to an origin of replication, which is a sequence of DNA talented to guiding the proliferation of it and any related sequence. Nevertheless, other facilities are required, and an assortment of specific cloning vectors exist that facilitate protein, single stranded RNA or DNA production and a host of additional molecular biology tools. Firstly, the target DNA requires to be isolated and purified to provide a DNA fragment of appropriate

Subsequently, a ligation procedure is used where the amplified fragment is inserted into a vector (piece of DNA). The vector (which is frequently circular) is linearized using restriction enzymes, and incubated with the fragment of interest under suitable conditions with an enzyme called DNA ligase. Following ligation the vector with the insert of interest is transfected into cells. A number of other techniques are available, such as chemical sensitization of cells, electroporation, optical injection and biolistics. At the end, the transfected cells are cultured.

As the forenamed techniques have a small proficiency, we require to recognize the cells that have become transfected positively with the vector molecule enclosing the favorable DNA segment in the correct orientation. Modern vectors which have widely used in biology contain an eligible antibiotic resistance indicator, which allow only cells in which the vector has been transfected, to grow. Additionally, these vectors may contain colour selection indicators, which provide blue/white screening on X-gal medium. However, these selectable markers do not undeniably warranty that the DNA segment is inserted correctly to the target cells. Additional study of the created colonies is mandatory to approve that cloning was effective. It can be done by the help of PCR, DNA sequencing, and also restriction fragment analysis.

3. Cell cloning

In the cell cloning, scientists are able to multiply a cell. This procedure is very simple and fundamentally just needs the inoculation of the suitable culture. Nevertheless, in the event that the cell will cultures from multicellular beings, cell cloning exclusively focuses on creating a population from a single cell. In the case of single cell organisms, the cultivation process is complicated due to the difficulty of growth of such cells in standard media.

A beneficial tissue culture method should be used to clone separate lines of cell lines includes the use of cloning rings (cylinders). In this method a unicellular suspension of cells that have been subjected to a mutagenic factor or drug applied to act as selection is plated at high dilution to generate isolated colonies, each growing up from a single and actually clonal separate cell. At an early growth phase when colonies comprise only a few cells, polystyrene rings, which have been dished in grease, are sited over a distinct colony and a small quantity of trypsin will be added. Replicated cells are collected from inside the ring and relocated to a new vessel for further growth.

4. Organism cloning

Organism cloning discusses about the practices of generating a new multicellular creature, genetically equal to another. In the wildlife this form of cloning is the non-sexual path of generation, where zygosis or inter-gamete interaction does not happen. Non-sexual proliferation is a normal pattern in many beings, comprising most plants and some bugs. Researchers could reach to valuable successes in order to cloning some animals with the help of non-sexual production procedures. There are numerous moral issues in the application of cloning. However, cloning or non-sexual proliferation has been exercised in the horticulture for hundreds of years.

4.1. Cloning Humans

The vision of cloning hominids is extremely debated, and it increases a number of moral, lawful, and public encounters that requires to be solved. Most of the researchers and politicians view humanoid generative cloning-cloning for creating a human baby- unethical. Advocates of the human cloning appreciate it as a likely answer to sterility difficulties. Some even visualize creating clones of geniuses, whose work could improve civilization. Unbelievable visions imaging farms stuffed with clones whose tissues are collected for transplantation-an actually terrible goal.

Till now, threats and methodical challenges-such as rules that make it unlawful-will possibly save humanoid reproductive cloning from becoming a reality. Albeit several beings have been cloned positively, the procedure is still theoretically challenging and unproductive. The achievement degree in cloning is relatively small: most germs fail to grow, and many gestations end with failure. Present endeavors at humanoid cloning are concentrated on generating embryonic stem cells for study and medication, as defined previously. Nevertheless, it feels that this kind of therapeutic cloning moves hazardously toward humanoid reproductive cloning.

4.2. Animal cloning

In reproductive cloning, scientists remove a developed somatic cell (e.g. a skin cell) from a being that they want to duplicate. They then relocate the DNA of the donor creature's somatic cell into an ovum cell that has had its peculiar DNA-comprising nucleus detached. Scientists can add the DNA from the somatic cell to the unfilled ovum in two dissimilar methods. In the first technique, they remove the DNA-comprising nucleus of the somatic cell with a stylus and insert it into the unfilled ovum. In the other technique, they use an electric shock to mix the whole somatic cell with the unfilled ovum.

In both methods, the ovum is permitted to grow into an early-phase embryo in the test tube and then is entrenched into the uterus of a mature female creature. Finally, the mature female gives birth to a creature that has the identical genetic structure as the animal that presented the somatic cell. This new animal is called a clone. Reproductive cloning might need to use of a successor mother to provide growth conditions for the duplicated embryo, as it used in the cloning process of the most well-known cloned organism, Dolly the sheep.

During the previous 50 years, researchers have directed cloning trials in a varied assortment of animals by a range of methods. In 1979, scientists created the first genetically equal mice by dividing mouse embryos in the test tube and then inserting the subsequent embryos into the uterus of mature female mice. Soon after that, scientists created the first genetically equal sheep, chickens and cows by removing the nucleus of a cell derived from an early embryo into an ovum that had been discharged of its nucleus. In 1996, scientists could be victorious in cloning the first mammal from a mature cell derived from a mature animal. Following 276 tries, scientists lastly created Dolly, the lamb derived from the udder cell of a 6-years-old sheep. After two years, scientists in Japan cloned 8 calves from a single cow, but unfortunately, only 4 stayed alive.

4.3. Artificial cloning

There are three dissimilar forms of artificial cloning: gene cloning, reproductive cloning and remedial cloning. Gene cloning generates duplicates of genes or pieces of DNA. Reproductive cloning generates duplicates of entire animals. Remedial cloning generates embryonic stem cells for investigates intended to producing tissues to interchange wounded or unhealthy tissues. Gene cloning, also known as DNA cloning, is a diverse procedure from two other cloning forms. Reproductive and remedial cloning have many common and similar procedures, but are used for dissimilar objectives.

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6. http://learn.genetics.utah.edu/content/cloning/whyclone/

4D BIOPRINTING FOR BIOMEDICAL APPLICATIONS

3D bioprinting has instituted the extensive use in numerous engineering and biomedical grounds; but, 3D bioprinting is inert and lifeless since it reflects merely the primary state of the printed item.

Newly, 'time' has been combined with 3D bioprinting as the 4th dimension, pretended '4D bioprinting', were produced substances (e.g., biocompatible reactive tools or cells) are talented of altering their forms or abilities with time when a peripheral motivator is inflicted. This improvement in printing reactive ingredients that can alter their shape, or ingredients that can rearrange with cellular self-association has widened the uses of 4D bioprinting in numerous biomedical arenas, such as tissue engineering and drug delivery.

4D Bioprinting

4D bioprinting is similar to 4D printing in that it is the printing of ingenious, ecologically reactionary to biological configurations, tissues and organs. 4D bioprinting initiates with the printing of various cells or biological mediums producing configurations that undertake the following, planned and predicted, but self-originated growth in reaction to a situation. Four different categories of 4D bioprinting have been projected. One form, called "shape change", represents a 4D printed substrate, collected from smart biopolymers (and cells) which alters its 3D formation (or figure) upon incitement. The second kind, named "size change", is known as a kind of *in vivo* bioprinting, in which a 3D printed apparatus is inserted in the body, and following biological actions permits tissue where the graft has once being present. In a third method, labelled "pattern change", microdroplets of cells are printed in a gathering of a specific design or direction. Comparable to 4D printing, the previous three kinds of bioprinting all comprise an engineered and foreseen altered in the combinations after-printing physical outline, dimension or arrangement.

Finally, in the last kind of bioprinting, 4D bioprinting comprises the exclusive constituent of a vibrant, biological improvement and reaction, in contrast with 3D printing.

In 4D printing a regulator can be closed and open, an airfoil can stoop and adjust, and a window can be closed and open. Nevertheless, in the 4D bioprinting setting, substantial practical and pre-engineered variations can

be intended to happen which are reliant on and/or end in non-structural but extremely biologically related actions. As an innovative method, 4D bioprinting could be a talented answer to unsolved medical requests, for example tissue renewal, and could catch extensive usage in the biomedical arena.

Biomedical applications of 4D bioprinting

4D bioprinting appeared as a valuable instrument for medical uses, such as tissue renewal and medicine transfer, because of its distinctive benefits as well as: (i) manufacture of 3D multifaceted tissue structures according to receptive resources that are talented to reform, or alter their action based on ecological motive; and (ii) after puberty of printed cell populations in frames with programmable designs, allowing the manufacture of tissue concepts with features that are identical to those of natural tissues. In following, the numerous instances of the use of 4D bioprinting in tissue manufacturing and drug delivery will be discussed.

1. Tissue engineering

New progresses in our perception of blood veins construction and the accessibility of artificial skills (e.g. 4D bioprinting) have meaningfully reformed the scenery of possible policies for creating biomimetic blood veins *in vitro*. For example, auto-flexible polymers can be applied to construct blood vessels by condensing diverse kinds of cell and may form multifaceted tube-like constructions in the attendance of water. An alternative technique to generate veins via 4D bioprinting is to take benefit of the normal self-arrangement capabilities of the cells. Vascularization is a crucial subject in tissue manufacturing that requests to be solved since there is a factual and critical necessity to engineered operational tissues.

Blood veins are the focal canals to preserve the cellular action by providing nutrients and oxygen; and also, eliminating excess produces. Commonly, bulk distribution is just an operative in providing tissues over a rout of 200 μm, which restricts the tissue to this dimension in the nonappearance of vascular transmission. 4D bioprinting affords a hopeful method to address this task. By the layer-by-layer bioprinting, the cells assorted in the hydrogels can be produced into cylinder-form configurations similar to the vasculature. For instance, manifold cell classes, as well as fibroblasts, MSCs and ECs were simulated in a hydrogel over a 4D printing method. Cell relocation and accumulation happened in a culture age of 16 days, causing in the development of vascular perfection, as verified by the expression of endothelial-peculiar genes.

In this circumstance, numerous aspects, as well as growth factors, EC–MSC relations, ECM constituents, mechanical elements, and hemodynamics can affect the development of a practical vascular system. Hundreds of lipid-covered watery drops have been produced in lipid and shaped constant bilayers.

3D tissue-similar structures can be simply manufactured via heterologous droplet gathering. Systems can also be automated by osmolarity gradients to wrinkle into wrapped constructions, which embrace the excessive possibility to construct vascular-similar tissue structures. One main challenge of these methods is their incapability to form vascular constructions on a slight range because of the imperfect clarity of the structures. Dissimilar to the mentioned plan, which practices the vasculature by remaining pressure and flex modulus, an alternative technique allows self-folding via a peripheral tautness applied by cells on the ECM or primary coating, identified as a cell tension power. 2D simulated micro plates roll in lengthways oblique lines into the cell tension power. So, the thickness of the cylinders can be adjusted by altering the angle of the diametric streaks. Tubular pipes have been constructed by bovine carotid vein endothelial cells and standard HUVECs to imitate vascular constructions.

4D bioprinting has similarly been approved to print hard-tissue structures (e.g. bone implants). At this point, a polymeric bone implant with a lattice design was printed and then covered with human rhinal quadratic turbinate tissue-imitative MSCs (hTMSCs) for implant mineralization. After a short cultural cycle, the printed bone material implant became completed, and the decellularized implant was examined both *in vitro* and *in vivo* and showed to have enhanced osteoinductive and osteoconductive attributes. Although, the bone implant was not as durable as native bone mechanically, and additional studies are required to enhance the rigidity of these produced bone tissue-engineered implants.

2. Drug delivery

4D bioprinting allows the accurate control of the 3D spreading of diverse constituents that can auto-fold or auto-unfold to condense and discharge medicines or cells in a programmable mode. For instance, tiny oil drops condensing watery droplets, named ‘multisomes’ may be printed in aquatic fluid. The watery droplets stick to one another and form boundary bilayers. Over modifications in temperature or pH of the nearby atmosphere, the constituents in the droplets can be discharged. Such a multi-chamber context of multisomes can afford as a small-sized bioreactor and has possible uses in medicine and/or cell delivery.

The multisomes can likewise be operational via membrane proteins. For example, a precise path was intended

to attain quick electrical connection among multisomes. The multisome systems can likewise be planned by osmolarity inclination, allowing them to fold and unfold into intricate constructions, causing the encapsulation and discharge of required fillings, with possible uses in drug delivery. Moreover, bioprinted auto-foldable or auto-unfoldable duplex constructions that can react to temperature alteration have been applied for yeast cell encapsulation and discharge.

Alternative technique similarly applied distinctly, uses polymeric hydrogel layers to form auto-folding machines, allowing the oriented encapsulation and discharge of medicines. This machine included two divers swelling layer and a mucoadhesive layer filled with medicines. The bilayer construction controlled two layers with dissimilar swelling proportion. One was a pH-sensitive hydrogel layer including cross-linked poly methyacrylic acid that swells meaningfully when in interaction with body liquids. Alternative layer was a non-swelling layer including poly hydroxyethyl methacrylate (PHEMA). The machine successively self-folded into the mucus because of the dissimilar swelling (ratio/rate) of the bilayers, which can meaningfully enhance the mucoadhesion. The PHEMA layer can similarly perform as a dispersal obstacle, reducing the medicine escape in the intestine. Bovine serum albumin and acid orange were condensed in the bilayered construction and applied for the fruitful unilateral discharge of medicines that then move in the mucosal epithelium.

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MINI-STOMACHS BREW INSULIN IN MICE

The modified tiny abdomens can someday supply insulin to persons with diabetes. Defect in cells, which generate insulin is an insignia of diabetes. Tissues in the lower stomach, renew mostly by native stem cells and often apply the similar genes found in pancreatic beta cells. By focusing on three main genes in cells of the lower stomach, researchers may could modify a feasible substitution for those beta cells. In investigations with genetically modified mice that lacked beta cells, modified stomach cells released insulin and glucose at standard levels in the blood. To make this method a practical treatment for people, the scientists acquired stomach stem cells from diabetic rats, modified them with similar genes and cultivated tiny tissues. Following, academics inserted the new tiny stomachs to the rat's body. 5 among 22 rats preserved standard glucose levels, and, their grafts contained some cells that secreted insulin. That portion might appear low; however, it demonstrates that the method might be exploited as a model for treatments that substitute beta cells in persons.

Reference: https://www.sciencedaily.com/releases/2016/02/160218132245.htm

BACTERIA TAKE MUG SHOTS OF THREATENING VIRUSES

Scholars from a number of universities worldwide, figured out that microbes comprise a structure that identifies and disturbs perilous viruses by a recently diagnosed workmanship relating to RNA. The finding might produce healthier approaches to neutralize viruses that harm agricultural yields and making of dairy crops. The scientists realized that bacteria can hijack some quantity of RNA from attackers, e.g. viruses, and integrate the RNA into their own genomes. This path may assist the bacteria, identify and disturb dangerous viruses in the forthcoming incidents.

Scientists similarly are studying how to genetically modify a crop so that their cells acquire this virus indicator. Alternatively use could be in the dairy productions, where viruses contaminate the bacteria that produce cheese and yogurt, triggering the procedure to hold up or avoid it from ending.

Reference: https://www.sciencedaily.com/releases/2016/02/160225153423.htm

GENETICALLY MODIFIED *E. COLI* PUMP OUT MORPHINE PRECURSOR

A popular abdomen microbe can quickly propose us a good paregoric. Scientists at Kyoto University and their coworkers have optimized *Escherichia coli* genes so that they give off thebaine, a morphine precursor that can be altered to create pain relievers. The genetically engineered *E. coli* procreates 300 times additional thebaine with a lesser danger contrast to a newly industrialized technique by yeast.

"Morphine has an intricate molecular assembly; owing to this, the manufacturing of morphine and comparable pain relievers is costly and time-consuming," said one of the researchers F. Sato of Kyoto University. "However, with our *E. coli*, we could harvest 2 mg of thebaine in a short time from approximately twenty grams of sugar, as contrasting with 0.0064 mg from yeast."

Morphine is elicited from poppy juice in a manner that yields opiates as well as codeine and thebaine. Other naturalists lately altered a yeast, thus it can secretes opiate alkaloids from sugar. But, there were moral concerns.

With *E. coli*, Sato mentions that the risk of such invention is low. "4 strains of genetically engineered *E. coli* are essential to change the sugar to thebaine. *E. coli* is more arduous to succeed and needs skill in management." In 2011, Sato and coworkers modified *E. coli* to produce reticuline, another morphine originator that emerges former in the conversion procedure than thebaine. In the novel method, the group added genes from other microorganisms and enzyme genes from *Opium poppies*, Arabidopsis, and *Coptis japonica*. The group attributes the powerful action of enzymes in the novel method for their achievement in generating thebaine, and hopes to attain additional developments. "By adding two additional genes, our *E. coli* were capable to produce hydrocodone, which would surely increase the feasibility of this method," Sato said. "With one or two more developments in the system and permission of pharmaceutical rules, engineering morphine-like pain relievers from microorganisms could shortly be an actuality."

Reference: https://www.sciencedaily.com/releases/2016/02/160225101103.htm

Book Alert

ASPERGILLUS AND PENICILLIUM IN THE POST-GENOMIC ERA

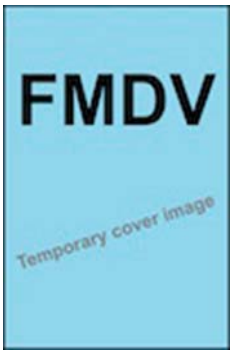
Publisher: Caister Academic Press

Editors: Ronald P. Vries, Isabelle B. Gelber and Mikael R. Andersen.

Publication date: 2016

ISBN: 978-1-910190-39-5

FOOT AND MOUTH DISEASE VIRUS: CURRENT RESEARCH AND EMERGING TRENDS



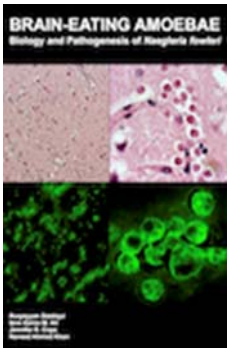
Publisher: Caister Academic Press

Editors: Francisco Sobrino and Esteban Domingo

Publication date: 2017

ISBN: 978-1-910190-51-7

BRAIN-EATING AMOEBAE: BIOLOGY AND PATHOGENESIS OF *NAEGLERIA FOWLERI*



Publisher: Caister Academic Press

Editors: Ruqaiyyah Siddiqui, Karim M. Ali, Jennifer R. Cope and Naveed A. Khan

Publication date: 2016

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Announcements

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http://adpd2017.kenes.com/

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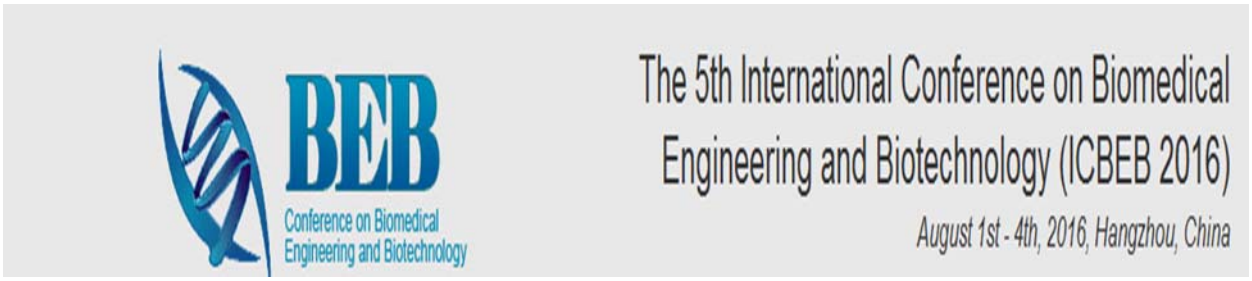
Announcements



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GLUCOSE UPTAKE WITH THE HELP OF INSULIN

Insulin interlocks to its receptor, which begins numerous protein stimulation cascades. These comprise displacement of Glut-4 transported to the plasma membrane and entrance of glucose, glycogen synthesis, and glycolysis in addition of triglyceride synthesis. The effects of insulin on the general human metabolism includes: the regulation of cellular consumption of specific materials, most importantly glucose in muscle and adipose tissue, enhance in the DNA duplication and protein production by regulating the amino acid absorption, and alteration in the function of various enzymes. Insulin also impacts other body actions, for example, vascular conformity and perception. As soon as insulin arrives to the human brain, it improves learning and memory and assists lingual memory especially. Insulin similarly has a stimulatory influence on gonadotropin-discharge from the hypothalamus, therefore supporting fertility.

Reference: https://en.wikipedia.org/wiki/Insulin

PENICILLIUM

Penicillium is a fungus and has a high value in the lifecycle, such as medicine and nutrition manufacturing. A medicine called penicillin can be generated by this fungus. This medicine can remove or halts the progress of bacteria and is known as antibiotic. Other classes of the fungi are employed in cheese production. Based on the dictionary of the Fungi, the general class comprises over 300 types. *Penicillium* is categorized as a genus of domain Eukaryota, kingdom Fungi, division Ascomycota. *Penicillium* classes are existent in the air and soil of enclosed surroundings, as well as houses and public structures. The fungus may spread from the outside, and propagate inside by building physicals or amassed soil to gain nutrients for development.

Reference: https://en.wikipedia.org/wiki/Penicillium

ORGANIC FOOD

Organic foods are nutriments manufactured by approaches that meet the terms of the standards of organic agriculture. Standards differ globally; nevertheless, organic farming in overall, includes observes that struggle to recycle the materials, boost environmental stability, and preserve biodiversity. Administrations modifying organic foods might select to limit the utilization of some insecticides and composts in agriculture. Overall, organic nutrients are usually not administered by radioactivity, industrial solutions or artificial food flavors.

Presently, some countries such as the European Union, the United States, Canada, Mexico, Japan, and many others necessitate fabricators to gain extraordinary authorizations to present nutriment as organic, inside their country. At the base of these rules, organic food is nourishment manufactured in a way that fulfills with organic ethics regulated by state administrations and universal societies. While the harvest of kitchen gardens might be organic, marketing food with the organic brand is controlled by legislative food security specialists, as well as the US Department of Agriculture (USDA) or the European Commission. There is no adequate indication in medical texts to backing statements that organic food is safer or better than common produced food. However, there might be some variations in the nutrient and anti-nutrient subjects of organic and commonly manufactured food, the flexible essence of food making and management makes it hard to simplify consequences. Titles that the natural food flavors are better are mostly not backed by documents.

Reference: https://en.wikipedia.org/wiki/Organic_food