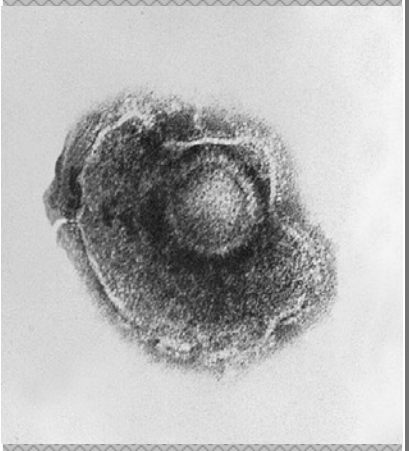

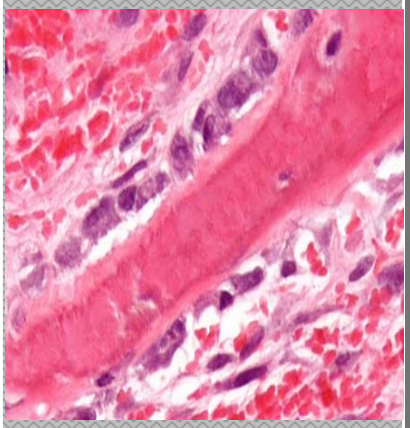

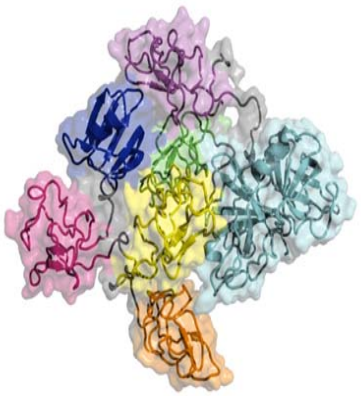

EMGEN Newsletter

Vol. 6, Issue 4

IN THIS ISSUE:

Eastern Mediterranean Health Genomics and Biotechnology Network (EMGEN) was created in 2004 with collaboration of representatives of selected centers of excellence in (health related) molecular biology, biotechnology & genomics in the Eastern Mediterranean region by recommendations and efforts of WHO/EMRO. Sponsored by Iran Biotechnology Development Council.

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HYBRIDOMA TECHNOLOGY

This procedure begins by vaccinating a mouse with an antigen that excites an immune reaction. A variety of WBCs, the B-cell, that generates antibodies are then gathered from the blood. These insulated B-cells are then attached to eternal B-cell tumor cells to generate a cross cell line named a hybridoma, which has both the antibody-generating capacity of the B-cell and the extreme durability and proliferation of the myeloma. The hybridomas can be developed in medium, every medium begins with one durable hybridoma cell, creating mediums with genetically equal hybridomas which yield one antibody per medium (monoclonal). The myeloma cell line that is utilized in this procedure is nominated for its capability to raise in tissue medium and for a lack of antibody creation. Counter to polyclonal antibodies, which are combinations of several dissimilar antibody fragments, the monoclonal antibodies created by every hybridoma line are all chemically equal. The creation of monoclonal antibodies was designed by C. Milstein and G.J.F. Köhler in 1975. They won the Nobel Prize in 1984 for Medicine and Physiology with N.K. Jerne, who helps in other aspects of immunology. The word “hybridoma” was invented by L. Herzenberg through his vacation in César Milstein's laboratory in 1977.

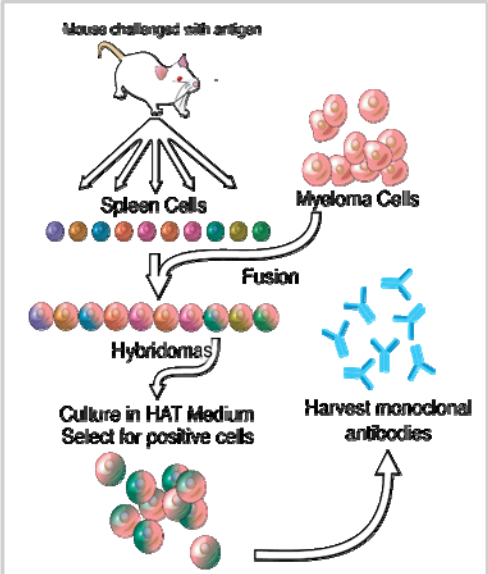

Figure 1: A general representation of the hybridoma technique utilized to produce monoclonal antibodies.

The methodology of hybridoma technique:

Experimental mammalian creatures (e.g. mice) are initially inoculated with an antigen that consequently, an antibody will be produced versus it. Typically this will be carried out via a chain of inoculations of the antigen, during a few weeks. These inoculations are usually trailed by the practice of *in vivo* electroporation, which meaningfully improves the immune reaction. As soon as splenocytes are insulated from the milt, the B-cells attach to eternalized myeloma cells. The combination of the B-cells with myeloma cells will be carried out by electrofusion. Electrofusion arranges and attaches B-cells and myeloma cells with the help of an electric ground. On the other hand, B-cells and myelomas can be attached by chemical substances, e.g. polyethylene glycol. The capable myeloma cells are chosen before to confirm they cannot produce antibody and also they have a shortage in the Hypoxanthine-Guanine PhosphoRibosyl Transferase (HGPRT) gene, which will

make them susceptible to the HAT culture. Attached cells are then incubated in HAT culture (Hypoxanthine-Aminopterin-Thymidine) for about 10 to 14 days. Aminopterin shuts the path that permits nucleotide production. Therefore, unattached myeloma cells demolish, as they cannot yield nucleotides by the *de novo* or recovery paths since they have deficiency in HGPRT. Elimination of the unattached myeloma cells is essential since they have the likelihood to fill out whole the culture, particularly in weakly settled hybridomas. Unattached B-cells demolish because they have a short life period. In this fashion, just the "B-cell-myeloma hybrids" stay alive, as the HGPRT gene in the B-cells is operational. These cells create antibodies (via B-cells) and are eternal (via myeloma cells). The incubated culture is then transmitted to multi-well dishes, somehow every well encloses merely one cell. As antibodies in a well are created by the similar B-cells, they will introduce same epitope, and are accordingly monoclonal antibodies.

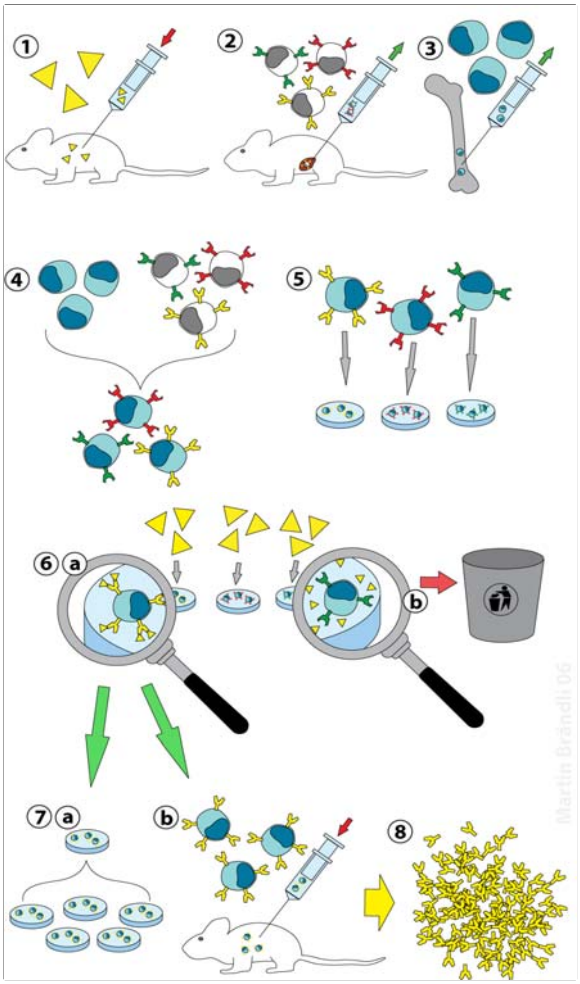

Figure 2: hybridoma technique steps:
(1) Immunization of a mouse.
(2) Isolation of B-cells from the spleen.
(3) Cultivation of myeloma cells.
(4) Fusion of myeloma and B-cells.
(5) Separation of cell lines.
(6) Screening of suitable cell lines.
(7) *in vitro* (a) or *in vivo* (b) growth.
(8) Harvesting hybridoma molecules.

The subsequent phase is a quick principal selection procedure, which recognize and chooses solitary those hybridomas that generate proper antibodies. The initially operated selection method is titled ELISA. The hybridoma medium will be prepared, marker enzyme is labeled by chromogenic material then incubated, and finally the creation of a dyed product specifies an affirmative hybridoma. Complementary cultures comprising interleukin-6 (e.g. briclone) are crucial for this phase. Otherwise, immunocytochemical selection can similarly be utilized. The B-cell that generates the favorite antibodies can be cloned to generate numerous equal copies. When a hybridoma colony is produced, it will incessantly develop in suitable mediums like RPMI-1640 and generate antibodies. Multiwell dishes are applied primarily to cultivate the hybridomas, and after selection, are displaced to greater tissue culture containers.

This preserves the well-being of the hybridomas and delivers sufficient cells for freezing and supernatant for succeeding studies. The medium supernatant can produce 1 to 60 µg/ml of monoclonal antibody, which is preserved at -20°C or lesser for desirable time.

Identification and isolation of the hybridoma cells

Hybridoma cells generating precise antibodies can be insulated by subsequent steps: 1) The precise antibodies existing in the every microwell are equal and can be identified by precipitation approach or agglutination approach. Most sentient, quick and frequently applied technique is ELISA (Enzyme Linked Immunosorbent Assay), 2) Wells which enclose the antibodies specific to the antigens are recognized and hybridoma cells are insulated from these wells and cultured, 3) After that, hybridoma cells are increased by *in vitro* or *in vivo* approach. This guarantees that these hybridoma cells have the capability to yield equal particular kind of antibodies specific to the antigen utilized.

Mass production of antibodies:

The *in vivo* approach includes inserting hybridoma cells into the peritoneal cavity of the target animal, then ascetic liquid is insulated and then antibodies are insulated from it. In *in vitro* approach hybridoma cells are cultivated in appropriate media and then antibodies are insulated and filtered.

Applications

The applications of monoclonal antibodies are various and comprises the avoidance, analysis, and cure of illnesses. For instance, monoclonal antibodies can differentiate subdivisions of B-cells and T-cells, which is useful in recognizing diverse kinds of leukemia's. Furthermore, precise monoclonal antibodies have been utilized to describe cell external indicators on WBCs and other cell types. These are usually stated as CD indicators and describe numerous dissimilar cell surface constituents of cells, each identified by binding to a specific monoclonal antibody. Such antibodies are exceptionally valuable for fluorescence-activated cell categorization.

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3. https://en.wikipedia.org/wiki/Hybridoma_technology

MESENCHYMAL STEM CELL

MSCs are an instance of 'immature' stem cells. MSCs are totipotent cells that can separate to a variety of cell classes comprising: adipocytes, myocytes, osteoblasts, and chondrocytes. This category has been acknowledged in definite cells and tissues in alive creatures and their equivalents rising in tissue medium. Other studies proposed that MSCs might similarly separate to numerous dissimilar kinds of cells that do not participate in the skeletal tissues; for instance, nerve cells, heart muscle cells, liver cells and endothelial cells, which make the internal cover of blood vena. In some circumstances, it seems that the MSCs might have attached together with particular cells, causing wrong decisions about the capability of MSCs to generate particular cell kinds.

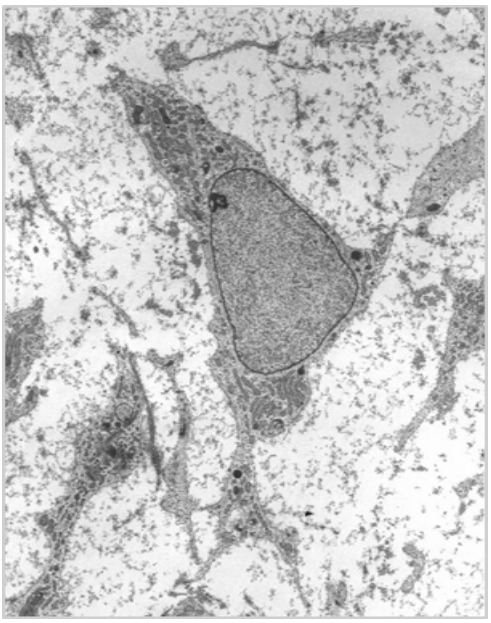

Figure 1: Mesenchymal stem cell.

In other circumstances, the consequences were a feigned outcome triggered by chemicals applied to cultivate the cells in the laboratory. MSCs were initially established in the bone marrow. There are several statements that MSCs furthermore occur in a wide range of other tissues, as well as umbilical cord blood, adipose tissue and muscle. The bone marrow comprises several different kinds of cells. There is blood stem cells and a range of different kinds of cells known as 'mesenchymal' cells. Solitary nearby 0.001 to 0.01% of the cells of bone marrow are mesenchymal stem cells. It is hardly possible to gain a combination of dissimilar mesenchymal cell kinds from mature bone marrow for study. Still, separating the small portion of cells that comprises mesenchymal stem cells is more difficult. Some of the cells in the combination might be capable to procedure bone or fat tissues, however, it does not have all the possessions of mesenchymal stem cells.

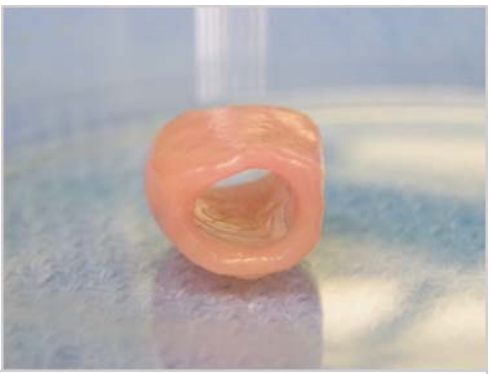

Figure 2: Typical gross appearance of a tubular cartilaginous construct engineered from amniotic mesenchymal stem cells.

Morphology

Mesenchymal stem cells are described morphologically by a tiny cell frame with a little cell developments that are stretched and cracked. The cell frame comprises a big, round nucleus with an eminent nucleolus, which is enclosed by excellently distributed chromatin fragments, giving the nucleus a perfect form. The residue of the cell frame comprises a slight quantity of Golgi apparatus, rough endoplasmic reticulum, mitochondria, and polyribosomes. The cells, which are stretched and cracked, are usually distributed and the nearby extracellular matrix is occupied by a small number of reticular fibrils but is empty of the other forms of collagen fibrils.

Immunomodulatory effects

Frequent investigations have revealed that humanoid MSCs evade allorecognition, intervene with dendritic cell and T-cell function, and create a local immune-repressive micro-atmosphere by discharging cytokines. It has similarly been publicized that the immunomodulatory act of humanoid MSC is increased when cells are subjected to an inflammatory atmosphere described by the attendance of raised local interferon-gamma rates. Further investigations deny some of these discoveries, mirroring both the extremely diverse nature of MSC isolates and the substantial variances among isolates created by the numerous dissimilar approaches in improvement.

Mesenchymal stem cells in treatment

1. Medical uses

The mesenchymal stem cells can be stimulated and mobilized if required. Nevertheless, the output is very small. For example, injury of muscles heals very andante but additional peruse of mechanisms of MSC act may deliver paths for enhancing their capability for tissue reparation. Numerous medical achievements using intravenous replacement have lead systemic illnesses such as implant against host illness and sepsis. Nonetheless, it is becoming more believed that illnesses, including peripheral tissues; for example, inflammatory bowel disease, may be better cured with approaches that enhance the local attentiveness of cells. Straight inoculation or settlement of cells into a position in the necessity of reparation may be the favored technique of cure, as vascular transport endures from a "pulmonary initial pass result" where intravenous inoculated cells are detached in the lungs. Medical case reports in orthopedic uses have been distributed, although the quantity of patients cured is small and these approaches still shortage precise studies representing usefulness.

2. Bone and cartilage repair

The capability of MSCs to separate to bone cells known as osteoblasts has caused their use in early medical surveys examining the security of possible bone overhaul approaches. These surveys are viewing the probable cures for localized skeletal deficiencies. Other examinations are focused on utilizing MSCs to overhaul gristle. Gristle protects the head of the bones and permits one bone to slip on another at the junctions. It can be injured by an accident like a crash, or during a long course of a chronic illness like osteoarthritis, a very hurting illness of the junctions. Gristle does not overhaul itself properly after an injury. The best cure accessible for acute gristle injury is surgery to substitute the injured joint with a dummy one. Since MSCs can separate to gristle cells known as chondrocytes, scholars expect MSCs could be inoculated to patients to overhaul and uphold the gristle in their junctions. Scholars are similarly examining the likelihood that transplanted MSCs may discharge ingredients that will force the patient's own cells to restore the injury. Several obstacles remain before this sort of cure can grow into a reality. For instance, when MSCs are transplanted, most of them are quickly eliminated from the body. Scholars are working on new approaches for transplanting the cells, as well as advance 3D-structures or frameworks that simulate the circumstances of the desirable part of the body where the cells are required. These frameworks will maintain the cells and force them to separate to the favorable cell type.

3. Medical studies of frozen MSCs

Researchers have informed that MSCs when transfused instantly during few hours post melting may display decreased activity or display reduced efficiency in curing illnesses in contrast to those MSCs which are in the log stage of cell growth, so frozen MSCs should be taken back to log stage of cell growth in *in vitro* medium before these are used for medical trials or empiric cures, re-culturing of MSCs will assist in retrieving from the shock the cells get through freezing and melting. Numerous medical studies on MSCs were unsuccessful which utilized frozen product directly post melting as compared to those medical studies which utilized fresh MSCs.

4. Inflammatory and autoimmune diseases

Numerous assertions suggested that MSCs are capable to evade recognition by the immune system and may be transplanted from one patient to another deprived of risk of immune refusal by the body. Nevertheless, these assertions have not been supported by other investigations. MSCs are banished similar to any other "non-self" cell type. Additionally, it proposed that MSCs might be capable to reduce the proliferation speed of

immune cells in the body to decrease inflammation and assist treat transplant refusal or autoimmune ailments. Once more, it should be confirmed and many more proofs are required to accept that MSCs could truly be utilized for this sort of uses.

5. Heart and blood vessel repair

Some examinations in mice propose that MSCs can stimulate the creation of novel blood vessels in a procedure named neovascularization. MSCs cannot create novel blood vessel cells; nonetheless, they might assist with neovascularization in some paths.

References:

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2. Phinney D.G. and Darwin J.P. (2007). Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair-current views . *Stem Cells*, 25(11): 2896-902.
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RESEARCHERS HARNESS DNA AS THE ENGINE OF SUPER-EFFICIENT NANOMACHINE

Academics at McMaster University have appointed a way to bridle DNA as a microscopic "machine" which can be switched on to discover trace quantities of materials, e.g. viruses, bacteria, cocaine, and metals. It's an entirely novel policy that can be adjusted to numerous types of applications, These DNA nano-structures are flexible, and hence any object can be measured. DNA is recognized as the genetic substance, however it is additionally a very programmable fragment that make it very capable for engineering in artificial requests. The new technique profiles distinctly programmed fragments of DNA into pairs of linking rings. The first ring stays passive until it is activated by the second, like a bike wheel in a lock.

When the second ring, performing as the lock, is subjected to even a trace of the objective material, it unlocks, releases the first ring of DNA, which duplicates rapidly and generates a sign, as well as a color change. “Biology utilizes all types of nanoscale molecular equipment to attain significant proceeds in cells", Li says. "For the first time, we produced a DNA-centered nano-apparatus that is talented to provide ultra-sensitive recognition of a bacterial pathogen". The DNA-centered nano-apparatus is under additional improvement to become a user-friendly recognition kit that will allow quick analyzing of a variation of constituents, and can achieve to medical trials within a year.

Reference: https://www.sciencedaily.com/releases/2016/07/160707083011.htm

GELATIN INSTEAD OF THE GYM TO GROW STRONGER MUSCLES

USC scholar M.L. McCain and coworkers have invented a way to produce larger, stronger muscle filaments. But as opposed to working on the arms of an athlete, these muscles cultivated on a tiny framework or "chip" shaped from a sort of water-filled gel prepared from gelatin. Through normal embryonic growth, skeletal muscles form when cells entitled myoblasts fuse to form muscle filaments, identified as myotubes. In previous trials, mouse myotubes have separated or exfoliated from protein-covered plastic frameworks after about one week and could not grow. In this study, scholars invented a gel framework from gelatin, a subsidiary of the naturally happening muscle protein collagen, and conquered very good outcomes. After 3 weeks, several of the mouse myotubes have been attached to these gelatin chips, and they were lengthier, broader and more advanced as an outcome.

The researchers expect that humanoid myotubes would develop similarly well on gelatin chips. This novel and enhanced "muscles-on-a-chip" can then be applied to study humanoid muscle growth and ailment, in addition to delivering a related analysis for new probable medicines. Tissue ailments such as skeletal muscle extremely decrease the excellence of life for millions of persons. By producing a cheap and available platform for investigating skeletal muscle in the lab, we expect novel cures for such diseases.

Reference: http://www.sciencenewsline.com/news/2016070205000032.html

HUMANS ARE NOT THE ONLY ONES WHO PRODUCE HALOGENATED ORGANIC POLLUTANTS

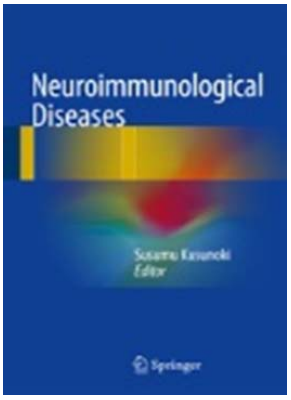
Scientists sieved the whole genomic catalog of a fresh jungle dirt in Tübingen, Germany to discover the variety, redundancy, and dispersal of germs skilled in halogenated biological mixtures alteration. The scientists found what they were searching for in bacteria, fungi and archaea, a sort of ancient bacteria. They revealed a formerly unidentified variety of genes coding halogenating enzymes in the dirt metagenome. It hypothesis that halogens are static and that most halogenated biological substance in dirt is anthropogenic have been faced by discoveries of naturally made organohalogens. While precise bacterial halogenation reactions have been identified for years and the connection among the dehalogenation of anthropogenic halogenated chemicals in lab cultures and polluted ground locations has been well recognized, just a few surveys definitely observed the fresh environments and the genetic potential for bacterial degradation of naturally happening organohalogens, Kappler states. In this novel research, they did not separate specific creatures from the jungle dirt, but they examined the entire gene pool found in the dirt, the metagenome.

They recognized all genes from bacteria, fungi and archaea coding for halogenating and dehalogenating enzymes. The most communal of these genes were related to those found in the types *Bradyrhizobium*, *Solibacter*, *Sphingomonas*, *Burkholderia*, *Mycobacterium*, *Mesorhizobium*, and *Pseudomonas*.

Reference: https://www.sciencedaily.com/releases/2016/07/160701094657.htm

Book Alert

NEUROIMMUNOLOGICAL DISEASES



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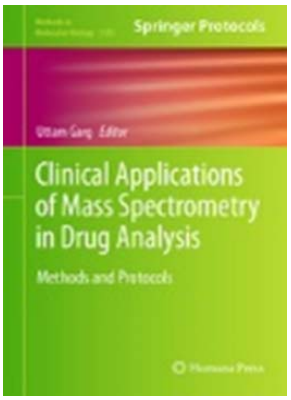
CLINICAL APPLICATIONS OF MASS SPECTROMETRY IN DRUG ANALYSIS



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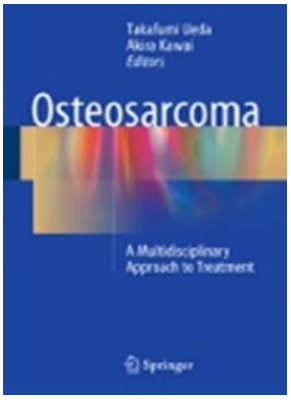

Publisher: Springer international publishing

Authors: Takafumi Ueda and Akira Kawai

Publication date: 2016

ISBN: 978-4-431-55695-4

Announcements

http://www.embl.de/training/events/2016/PRO16-02/index.html

http://www.icebs.org/

http://iastem.org/Conference/Greece/2/ICMBPS/

Announcements

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VARICELLA ZOSTER VIRUS

Varicella Zoster Virus (VZV) is a herpesviruse which infects humanoids. VZV usually causes chickenpox in all ages and herpes zoster (shingles) in adults and infrequently in kids. VZV is recognized by numerous terms, comprising zoster virus, varicella virus, human herpesvirus type 3 (HHV-3), and chickenpox virus. VZV grows in the lungs, and causes a wide range of signs.

Following the early infection (chickenpox), the virus goes latent in the nerves, as well as the cranial nerve ganglia, dorsal root ganglia, and autonomic ganglia. Several years after that patient has ameliorated from chickenpox, VZV can restart to induce several neurologic disorders. Early VZV infection causes chickenpox (varicella), which can cause problems including encephalitis, pneumonia or bronchitis.

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

OSTEOBLAST

Osteoblast are cells with a distinct kernel that produce bone. On the other hand, in the procedure of bone creation, these cells act in clusters of attached cells. Single cells are not capable to produce bone, and the cluster of prearranged osteoblasts alongside the bone produced by a group of cells is typically named the osteon. Osteoblasts are specific, ultimate matured arrangement of mesenchymal stem cells. They produce very compact, cross-linked collagen, and numerous other particular proteins in smaller amounts, comprising osteocalcin and osteopontin, which combine the organic matrix of bone. In prepared clusters of linked cells, osteoblasts produce a calcium and phosphate-based mineral, hydroxyapatite that is entrusted in an extremely controlled way, into the organic situation creating a very resilient and compact mineralized tissue - the mineralized matrix. The operational part of bone, the bone matrix, is completely extracellular.

The bone matrix comprises of protein and mineral. The protein is named the biological matrix; it is created first, and following the mineral is added. Most of the biological matrix is made from collagen, which delivers elastic strength. Then the matrix is mineralized by sedimentation of a calcium-phosphate-hydroxide salt entitled hydroxyapatite. This mineral is very firm, and delivers condensed strength.

Therefore, the collagen and mineral together are a complex material with perfect elasticity and condensed strength. Powers that surpass the ability of bone to act elastically may cause defaults, e.g. bone breaks.

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

PLASMIN

Plasmin is a vital enzyme extant in blood that destroys several blood plasma proteins, comprising fibrin clots. The elimination of fibrin is called fibrinolysis. In humanoids, the plasmin protein is produced by the PLG gene. Plasmin is a serine protease that operates to eliminate fibrin clots. In addition to fibrinolysis, plasmin proteolysis proteins in numerous other systems: It stimulates collagenases, some intermediates of the complement system and attenuates the wall of the Graafian follicle. It slices fibrin, fibronectin, thrombospondin, laminin, and von Willebrand factor. Plasmin, similar to trypsin, is a member of the serine proteases family. Plasmin is discharged as a zymogen entitled plasminogen from the liver into the blood. Two main glycoforms of plasminogen are existing in humanoids - type I plasminogen comprises two glycosylation halfs while type II plasminogen comprises just a single O-linked. Type II plasminogen is favorably employed to the cell surface above the type I glycoform. In opposition, type I plasminogen happens more frequently on blood clots. Plasmin is disabled by proteins like α2-macroglobulin and α2-antiplasmin. The mechanism of plasmin disabling includes the incision of a α2-macroglobulin. This starts a structural alteration such that the α2-macroglobulin breakdowns on plasmin. Following that in α2-macroglobulin-plasmin composite, the active site of plasmin is severely secured, therefore it significantly reducing the plasmin's accessibility to protein substrates.

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