Biyomühendislikte Güncel Yaklaşımlar:

Hücre Kültürü, Akıllı Sistemler ve Kanser Biyolojisi

Editör
HİKMET YETER ÇOĞUN

BİDGE

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ÖNSÖZ

Bu kitap, biyomedikal mühendisliğin güncel yönelimlerini laboratuvar temelli biyolojik yöntemler, akıllı/otonom sistemler ve yapay zekâ, moleküler onkoloji eksenlerinde bir araya getirerek disiplinler arası bir çerçeve sunmayı amaçlamaktadır. Hücre kültürü; ilaç geliştirme, hastalık mekanizmalarının aydınlatılması ve doku mühendisliği gibi alanlarda temel bir araştırma altyapısı oluştururken, standartlaştırılmış protokoller ve kalite kontrol yaklaşımlarıyla deneysel verinin güvenilirliğini artıran kritik bir yöntemdir.

Kitabın ikinci bileşeni, sağlık gibi insan yoğunluğunun yüksek olduğu ortamlarda güvenliği artırmaya yönelik ROS tabanlı akıllı sistemler yaklaşımını ele alır; poz tahmini ve derin öğrenme yöntemleriyle insan davranışlarının gerçek zamanlı tespitine dayanan çözümler, otonom sistemlerin çevresel farkındalığını güçlendirerek kritik senaryolarda karar destek kapasitesi kazandırır.

Üçüncü bileşen ise kolon kanserinin yayılımında belirleyici olan EMT/MET döngüsü, inflamatuvar sinyalleme, epigenetik düzenlemeler ve miRNA aracılı gen kontrolü gibi mekanizmaları bir arada değerlendirerek, translasyonel araştırmalar için hedef odaklı bir perspektif sağlar.

Bu eser; biyomedikal mühendisliği, moleküler biyoloji, sağlık bilişimi ve ilgili alanlarda çalışan araştırmacılar, lisansüstü öğrenciler ve uygulayıcılar için hem kavramsal bir bütünlük hem de güncel uygulamalara uzanan bir yol haritası sunmayı hedeflemektedir. Kitabın hazırlanmasında emeği geçen tüm yazarlara, hakemlere ve bilimsel üretimi destekleyen kurumlara teşekkür eder; çalışmanın literatüre ve uygulamaya katkı sağlamasını dileriz.

Prof. Dr. Ferit KARGIN

ÇUKUROVA ÜNİVERSİTESİ

İÇİNDEKİLER

BÖLÜM 1

Kolon Kanseri Yayılım Mekanizmalarında Epigenetik ve miRNA Değişiklikleri

Ayla Eren Özdemir

1. Giriş

Kolon kanseri dünya genelinde yüksek mortalite oranına sahip maligniteler arasında yer almakta olup tümörün metastaz yapma kapasitesi hastalığın seyrini belirleyen en kritik faktörlerden biridir [1]. Metastatik yayılımın temel biyolojik süreçlerinden biri olan epitel-mezenkimal geçiş (EMT), kanser hücrelerinin epitel özelliklerini kaybederek daha invaziv ve hareketli bir fenotipe geçmesini sağlamaktadır [2]. Bu süreçlerin geri dönüşümü olan mezenkimal-epitel geçiş (MET), kolonizasyon ve metastatik odakların oluşumunda önemli rol oynamaktadır [3]. Son yıllarda özellikle IL-6 gibi inflamatuvar sitokinlerin EMT'yi tetikleyerek metastazı hızlandırdığı gösterilmiştir [7]. Bu kitap bölümünde kolon kanserinin yayılım mekanizmaları epigenetik düzenlemeler ve miRNA aracılı gen kontrolü üzerinden ayrıntılı biçimde ele alınmaktadır.

2. Epigenetik Mekanizmalar

Epigenetik düzenlemeler, DNA dizisi değişmeksizin gen ekspresyonunun kontrol edilmesini sağlayan biyokimyasal süreçlerdir. Kolon kanserinde en çok çalışılan epigenetik mekanizmalardan biri DNA metilasyonudur; özellikle tümör baskılayıcı genlerin promotör bölgelerindeki hipermetilasyon, bu genlerin susturulmasına ve tümör progresyonunun hızlanmasına neden olmaktadır [20]. Histon modifikasyonları da kromatin yapısında gevşeme veya sıkılaşmaya yol açarak transkripsiyonel aktiviteyi düzenlemektedir. EMT'nin ana düzenleyicilerinden olan PRRX1'in epigenetik olarak baskılanması, metastazın erken evrelerinde kritik rol oynamaktadır [4]. ZEB1 ve ZEB2 gibi transkripsiyon faktörleri, E-kaderin gibi epitel belirteçlerini baskılayarak mezenkimal fenotipin oluşmasına katkıda bulunur [5]. LncRNA'lar da epigenetik yeniden programlamanın önemli bileşenleri arasında yer almakta olup HOTAIR gibi moleküller kromatin modifikasyon komplekslerini yönlendirerek metastatik progresyonu artırmaktadır [22].

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3. miRNA Düzenlenmesi

miRNA'lar yaklaşık 18–25 nükleotid uzunluğunda küçük RNA molekülleri olup post-transkripsiyonel gen düzenlemede görev almaktadır [11]. Kolon kanserinde miRNA-200 ailesinin EMT sürecindeki kritik rolü iyi bilinmektedir; bu ailedeki miRNA'ların baskılanması, ZEB1/2 aktivasyonunun artmasına ve epitel fenotipin kaybına yol açmaktadır [6]. Lin-4 ve let-7 gibi erken keşfedilmiş miRNA'lar hücresel zamanlama mekanizmalarını düzenlemekte olup kanser hücrelerinde farklı ekspresyon paternleri sergileyebilmektedir [11,12]. İnflamatuvar mikroçevre kaynaklı IL-6/STAT3 aktivasyonu, miRNA ekspresyon profillerini değiştirerek EMT'nin sürdürülmesine katkıda bulunmaktadır [21].

4. Materyal ve Metot

Bu bölümde DLD1 kolon kanseri hücre hattı kullanılarak IL-6 indüksiyonu ile EMT süreçlerinin tetiklenmesi amaçlanmıştır. Hücre kültürü standart protokollerle gerçekleştirilmiş, IL-6 maruziyeti sonrası RNA ve miRNA izolasyonu yapılmış, ardından gen ekspresyon düzeylerini analiz etmek amacıyla qPCR uygulanmıştır. Ayrıca epigenetik değişiklikleri incelemek üzere DNA metilasyon profilleri değerlendirilmiştir. Elde edilen veriler istatistiksel olarak analiz edilerek EMT/MET süreçlerinin moleküler temelleri ortaya konmuştur.

5. Bulgular ve Tartışma

IL-6 ile uyarılan DLD1 hücrelerinde EMT'ye özgü fenotipik değişimler gözlemlenmiştir. E-kaderin ekspresyonunda azalma, N-kaderin ve vimentin düzeylerinde artış tespit edilmiş, bu durum EMT'nin gerçekleştiğini doğrulamaktadır [2,17]. miRNA analizleri, miRNA-200 ailesinin belirgin biçimde baskılandığını göstermiştir [6]. Bu sonuç ZEB2 artışıyla uyumlu olup hücrelerin invaziv fenotipe geçişine katkı sağlamaktadır [5]. Epigenetik analizlerde PRRX1 ve bazı tümör baskılayıcı genlerde hipermetilasyon saptanmış, bu bulgu epigenetik baskılamanın metastazdaki rolünü desteklemektedir [4,20]. Ayrıca IL-6/STAT3 aktivasyonunun EMT'yi güçlendirdiği ve miRNA–epigenetik etkileşimlerinin metastatik yayılımı artırdığı görülmüştür [7,21].

6. Sonuç

Bu kitap bölümünde kolon kanserinin yayılım sürecinde epigenetik düzenlemelerin ve miRNA değişikliklerinin kritik etkileri kapsamlı biçimde ele alınmıştır. Elde edilen bulgular, EMT/MET döngüsünün epigenetik ve post-transkripsiyonel mekanizmalarla sıkı biçimde kontrol edildiğini göstermekte, inflamatuvar sitokinlerin bu döngüde düzenleyici

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rol oynadığına işaret etmektedir. Bu mekanizmaların daha iyi anlaşılması, yeni terapötik hedeflerin belirlenmesine katkı sağlayabilir.

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BÖLÜM 2

cell culture

Ahmet ALBYRAK[(1)]

I. Introduction

Cell culture refers to the cultivation of cells in vitro, or in a laboratory, under controlled conditions, while preserving their ability to function normally. Cell culture has numerous applications in virus research, vaccine production, studying gene function, and drug development. It is a valuable tool in basic research, disease mechanism investigations, and cell biology. Selectivity in cell culture relies on availability, cost, ease of handling, and speed. Besides, the application of standard cell populations and media provides enhanced reproducibility of data. For purposes of maintaining data accuracy, quality control is conducted regularly in the mycoplasma testing, karyotyping, and pluripotency and differentiation assays. Cell culture is a reliable technique for scientific study, drug discovery, and clinical investigations. Adherent cells grow in a monolayer at the bottom of the culture vessel. Representative examples include cells of human beings (Homo sapiens), house mice (Mus musculus), primates, and brown garden snails (Cornu aspersum). Cells can either be adherent or suspended.

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Adhesive cells adhere to the vessel surface and form a monolayer. Suspension cells drift around in the culture medium and never adhere to the vessel surface. Insect cells, modified human cells, and human blood cells are typical examples. Cells grow at temperatures ranging from 27°C (e.g., some fish cells) to 37°C (e.g., human cells), depending on their kind. Hücreler, in vitro koşullar altındaki her zaman ulaşılabilir ya da sıvı nitrojen kulu içinde dondurularak uzun bir süre dayanabilir. Although standard two-dimensional (2D) cell culture is the norm for most cell lines, three-dimensional (3D) culture systems that allow cells to grow in a more natural condition, e.g., the extracellular matrix (ECM), are becoming increasingly favored. They provide more realistic models. Cells can be cultured in various formats, ranging from low-volume 384-well plates to large-scale (>10 liters) production or high-throughput screening vessels. Automated liquid handling Systems are becoming increasingly popular, as they can introduce precision and consistency with minimal human intervention for large-scale screens or cultures.

The historical development of cell culture

Microscopes are rated as one of the most important tools that have helped in the development of biological sciences. They were important inproduction and utilization in nations like the Netherlands and Italy during the 16th and 17th centuries. In 1590, Dutchman Hans Janssen and his son created a compound microscope made of two converging lenses. At the same time, the famous Italian scientist Galile Galilei made a simple microscope andtelescope around 1610, naming itthe"occhialino." Italian physici an Giovanni Faber first utilized the word "microscope" in 1625.
The introduction of these discoveries and inventions has been instrumental in the progress of biological sciences, most especially in the field of cell culturing.

Robert Hooke and Antonie van Leeuwenhoek greatly contributed to microscopy in the early 17th century. The use of the microscope by these researchers led to the achievement of revolutionary findings. In 1665, Hook wrote the book titled "Micrographia," where he described the drawings and observations of several objects that he had examined with a microscope. In this book, the writer elaborates on how different entities, such as tirtol kelleri (tirtol cells), bitki dokular (plant tissues), and böcek kanı (insect blood), are seen under the microscope. In this explanation, the writer attempts to offer proof of the existence of the microscopic world.

Van Leeuwenhoek's work in microbiology led to the discovery of microscopic organisms that no other scientist had seen before. The scientist used a Mikroskop to see the existence of tiny organisms in water samples, in addition to distinguishing characteristics of cells found in blood and spermatozoa. Additionally, he is considered to be the first person to have discovered microorganisms.

It was the work of these two scientists that helped us learn more about the world at the micro level. The use of the microscope resulted in immense contributions to our knowledge of the microscopic world, thus proving its existence. It can be seen here that the above elements have been instrumental in laying the groundwork for many scientific fields, such as, but not limited to, medicine, biology, and chemistry[2].

In the year 1665, the English physicist Hooke published the first significant study on the construction of the microscope, its components, and microscopic observations. In Micrographia, numerous biological specimens (e.g., insects, plants, sponges, bryozoans, and fossils) were observed and characterized through the use of a microscope. The term "hücre" or "gözenek" is used to denote the microscopic structures in question. The study's author has selected the smaller divisions of a fungal cell (i.e., the thickened cell walls of dead cells) to illustrate these structures. The term "cell," as

employed by Robert Hooke in his Micrographia, was subsequently adopted by biologists and continues to be utilized extensively by contemporary biologists. The origin of the term "cell" can be traced back to Hooke's seminal work. [2], [3], [4].

The early 20th

century was the time when engineering principles of in vitro culture of plant and animal cells were established [4].The analysis of cellular culture information has become possible only with the use of the suspended drop cultivation technique. The tremendous impact on the advancement of cellular cultivation was due to the use of aseptic technique and the Rous and Jones tissue triplication technique[4], [5], [6].

Rous and his colleague found that the use of a trypsin solution allowed obtaining a single cell suspension and separating cells for subculture. 3% trypsin solution was successfully used for plasma digestion and did not damage most cells. When 5% trypsin solution was tested, the cells obtained died. Until then, cultures were obtained from tissue explants, and the use of trypsin facilitated the procedure to obtain homogeneous cell types [5] [7].

The “L” cell line is known as the first immortalized cell line and was generated in 1948 by American biologist George Gey at Johns Hopkins Hospital in Maryland for use in medical research. This cell line is derived from subcutaneous mouse tissue and is named “L” because the cells are found in the lymphatic tissue of the mouse skull. “L” cells divide rapidly and can differentiate in a similar way to tumor cells, characteristics that have helped researchers learn more about cancer cells [4] [6].

Cell lines are populations of cells created through cell culture and are distinguished from primary cell cultures. Primary cell cultures

consist of cells obtained directly from tissues or organs and are called “primary” until the first passage. Primary cell cultures are usually derived from normal or cancerous adult tissues or embryonic tissues. In primary cultures prepared using enzymatic and/or physical methods, the cell population is mixed and contains different cell types. Such cultures are used in many fields such as physiology and cellular metabolism, cytogenetics, pharmacology or tissue engineering [4] [8].

The subculture technique produces cell lines with serially subcultured cells from primary cell cultures. Even though cell lines produced from normal tissues have restricted growth, cell lines derived from cancer tissue grow without limitation. Normal cells also show indefinite proliferation through spontaneous transformation. Even though different cell lines are used in different useful studies, the use of cell lines is limited and not helpful in different aspects of drug development.

Scientists in the 1920s, such as Pannett and Compton (1924), Gay (1936), Earle (1943), and Hank's salts (1948), began to define the composition of salt solutions specifically formulated for cell culture. This was the start of defining the requirements of cell cultures and helped establish the components of utmost priority for cellular metabolism, i.e., amino acids, salts, vitamins, hormones, and glucose.

60 chemically formulated media have been explored between 1932 and 1962 [9]. Morgan, Morton, and Parker, for example, pioneered the development of media such as Essential Medium (EM) and Dulbecco Modified Eagle's Medium (DMEM) with essential and non-essential amino acids [10]. Media have been categorized into organ culture media and cell culture media, such as Trowell's T8 medium (1959) for long and short-term growth [9] [10].

on different scales (small and large scale). Large-scale cell culture development led to the creation of the Salk vaccine for polio infection. Poliovirus was cultured in simian and human kidney cells [10]. Today, cell culture media are often supplemented with antibiotics, but the first effect of antibiotics on cells cultured in vitro appeared in the 1940s. Herrell and colleagues found that different penicillin preparations showed toxic effect on mitosis due to some impurities in the penicillin preparation. Compared to penicillin G, it was practically harmless to cells [11] [12].

Keilova presented the direct effect of streptomycin on the heart, aorta and anterior bone explants of chick embryos [13]. Lawrence also found that high concentrations of antibiotics (including penicillin, streptomycin, tetracycline and neomycin) not only affected the migration of the epithelium around skin explants, but also caused respiratory damage or necrotic changes at some concentrations [12]. In another study, Krueger analyzed the effect of streptomycin on protein synthesis in mammalian cells and found that this antibiotic altered the in vitro synthesis of antibody against phage MS-2 in spleen and lymph node cells from immunized rabbits [14]. Furthermore, the culture medium requirements, physiochemical conditions for cell cultures must be properly fulfilled. The incubator was first used by Robert Koch in his microbiological studies in the second half of the nineteenth century. Incubators were also used by Virchow, Pasteur or Pettenkofer in their pioneering work. The use of incubators for cell cultures was proposed by Carrel and Burrows. When working with cell cultures of “warm-blooded” animals, they needed to maintain the appropriate culture temperature [15]. Previously, some scientists used only warm media to work with in vitro cultures, but this method was very inefficient. CO2 incubators became widely available commercially in the 1960s.l Today, cell culture is maintained in automated incubators that provide appropriate environmental conditions (temperature, humidity and

gas atmosphere). Most mammalian cell cultures require a temperature of 37°C, CO2 in the range of 5-10% and 95% relative humidity (RH) to minimize media evaporation and condensation. [15][16]. The growth of animal and human cell cultures, their cultivation, and the study of how they may be protected from fungal infection are the foundation for microbiological activity. In 1909, the W. K. Mulford Pharmaceutical Co. of Glenolden, Pa., marketed the first safety cabinet, a ventilated hood. The initial safety cabinet (a ventilated hood) was designed to protect against Mycobacterium tuberculosis infection while preparing tuberculin. Microbiological cabinet developments were made in 1943 in the Old World. Van den Ende constructed a safety cabinet featuring an electric furnace that generated airflow and recirculated exhaust air. Laminar tank cleanrooms with extreme or vertical registers of air were used in the 1960s. Cleanrooms were used in hospital environmental control and medication administration. Cleanrooms enabled the extension of filtered air over workpieces and technicians. Particulates in the air were trapped in HEPA filters, but the cleanrooms formed were expensive and not as portable as intended. Accordingly environment of Class II safety cabinets. Class II safety cabinets (laminar flow hoods, biological safety cabinets, BSCs) are primarily used to have a good environment and the experiment [94, 95], i.e., biological material while culturing cells, subculturing, and microorganisms. Laminar flow hoods are now equipped with HEPA (high-efficiency disease) filter through which diseases present in the air blown into the hood are eliminated. The cabinets also incorporate ultraviolet light (UVC, between 290 and 200 nm), which sterilizes the work surface of the hood. [17] [18].

1-Hayflick Phenomenon

The Hayflick phenomenon suggests that normal somatic cells have a limited lifespan and become non-dividing after a limited number

of divisions. It is linked to cell aging and damage, and suggests that normal cells have a limited lifespan.

He learned that from the work of Hayflick (1928) and Paul Moorhead, who demonstrated that normal human cells do have a limited lifespan. Hayflick conducted some research himself that was provoked by the work of Carrel as he began to investigate possible viral causes of human cancer. Hayflick exposed normal human embryonic cells to reagents made from cancer cells in 1958 in the hope that the normal cells would be changed and acquire cancerous characteristics. What occurred was the normal cells lost their capacity to divide. Hayflick thought he had gotten something wrong when he was making the medium, how he was sterilizing the glassware or whatever protocols he had set up. Several years later, in 1961, Hayflick worked with the cytogeneticist Paul Moorhead and they carried out a series of experiments that confirmed Carrel's initial hypothesis. Hayflick noted that normal somatic cells like human fetal lung cells, which were cultured, would stop dividing after a few divisions, which suggested that cell division would need to be limited finitely and that cells would eventually die. [19].

Cells' DNA sequences, specifically telomeres, shorten over time. Telomeres are repetitive DNA sequences located at the ends of chromosomes and diminish as cells replicate. After a certain number of cell divisions, telomeres become too short to allow further division, leading to cell senescence or death [20].

The Hayflick phenomenon is not found in some cells with unlimited ability to divide, such as cancer cells. Such cells possess an enzyme named telomerase that can extend their telomeres and thus divide again and again without stopping. The Hayflick phenomenon is crucial for studying human aging and age-related diseases. Understanding this process may help in the explanation of normal

cell aging causes and how treatments may be developed to potentially ameliorate the aging process [21].

2-HeLa cell line's immortality

In 1951, Henrietta Lacks, at Baltimore's Johns Hopkins Hospital, was diagnosed with an aggressive cervical adenocarcinoma. The treatment samples were submitted to Doku Kültürü Laboratuvarı Direktörü Dr. George Gay (1917-1994)[22]. Mary Kubicek, the assistant of Dr. Gay, put the samples in a turkey plasma-containing nutrient broth and observed that the cells survived. These cells were subsequently cultured in silinder tubes, where they developed vigorously, became healthy, and were divided every 20 hours. These cell cultures eventually became a HeLa-named cell line derived from the patient's name.

HeLa cells, due to their growth capacities and resistance, became very common in medical research. However, HeLa cell-related procedures were based on wrong information for years. Actually, the cells were even interpreted as coming from a different source, e.g., Harriet Lane or Helen Larsen[23], [24], [25]. Such HeLa cell source-based secret information was also related to these procedures, and in 1971, Women's Diseases and Obstetrics revealed the source of HeLa cells, Henrietta Lacks. This has caused an ethical dilemma between Lacks' family and the use of HeLa cells [26], [27].

In 1952, Dr. Gay and his colleagues published the results of one-year HeLa cultures. They stated that they had established and maintained "continuous cylinder tube cultures for almost a year." HeLa cells were shown to grow in a variety of media, including chicken plasma medium, bovine embryo extract, and human placental cord serum[23], [26], [27], [28].

The HeLa cell line established by Gay enabled Jonas Salk and John Enders to develop poliovirus cultures in a tissue system outside the

nervous system. Poliomyelitis virus was successfully propagated by Dr. Gay in HeLa cell cultures. The HeLa cell line was cultured in virtually every known culture medium and was quickly distributed to laboratories and cancer scientists in the United States and other countries. The HeLa cell line was also distributed to pharmaceutical companies, and HeLa cells became a popular and valuable resource for cancer research [[18], [23], [25], [29].

The most famous cell line, particularly the mechanisms that make it so aggressive, has been intensively studied. It is now known that HeLa cells are infected with human papillomavirus 18, which disrupts the p53 tumor suppressor gene protein responsible for protein synthesis. HPV18-positive HeLa cells exhibited changes in microRNA expression. [4], [23].

The HeLa genome was completely sequenced and made public in 2013 without notifying Lack's family members (the family later provided limited access to the HeLa genome information) [27]. Researchers from the European Molecular Biology Laboratory and the Institute of Human Genetics (Heidelberg, Germany) identified the HPV18 insertion on chromosome 8. The result concurred with previous observations, but nine additional putative viral integration points were discovered. Four of the HeLa chromosomes were also broken and reassembled into highly rearranged chromosomes. The process was termed "chromothripsis" and was identified to occur in 2–3% of all cancers. Chromothripsis has also been shown to occur, that is in chromosomes [30], [31]. Rearrangements in chromosomes 5, 19, and X have also been reported. Chromothripsis happens through the high CN crossovers, high interconnectivity, and low CN number alternations (2–3). Compared to normalized expression levels of gene expression of HeLa transcriptomes from Illumina Human BodyMap 2.0 of 16 tissues, 1907 genes of which 805 code for proteins, were found to have increased expression in HeLa cells.

Finally, 23,966 genes of which 5593 are coding for proteins were de-expressed in HeLa cells [[30], [31], [32], [33], [34].

According to findings put forward by Landry and colleagues, biologist Van Valen's 1991 speculation that HeLa cells have evolved into a new species—Helacyton gartleri—by multiple cell passages, viral infection, or other cell line contaminants no longer appears to have lost its relevance[35], [36].

3-İlk monoklonal antikorlar

The development of the first monoclonal antibodies is an important milestone in immunology and biotechnology. The first monoclonal antibodies were produced in 1984 through the groundbreaking work of César Milstein and Georges Köhler, and their collaborator Niels Jerne [37].

Milstein and Köhler published a seminal paper describing a technique for producing monoclonal antibodies. They describe the derivation of a series of cultured cell lines capable of secreting antibodies against sheep red blood cells (SRBC). The cell lines were obtained using mouse myeloma and mouse spleen cells from an immunized donor. Two myeloma cell lines derived from BALB/c mice were used for the cell combination. The P1Bu1 cell line was resistant to 5-bromo-2′-deoxyuridine and did not grow in HAT-selective medium. Thus, the cell line secreted a myeloma protein-IgG2A. The second cell line was P3-X63Ag8, which was derived from 8-azaguanine-resistant P3 cells and did not grow in HAT medium. P3-X63Ag8 secreted MOPC 21-IgG1(κ). Cell fusion was performed using inactivated Sendai virus. The karyotype of the hybrid cells (after 4 months) was lower than the sum of the two parental cell lines. After cell fusion with Sendai virus, cells of the P3-X63Ag8 line were able to grow in HAT medium and secreted immunoglobulins containing MOPC 21 protein [38], [39].

Monoclonal antibody manufacturing has transformed a vast array o f areas of science and medicine. Monoclonal antibodies have a wide range of applications in diagnostics, research reagents, and therapeutics. They are used for specific antigen detection in diagnostic tests, elucidation of cellular processes, and targeted therapies for an array of diseases such as cancer, autoimmune conditions, and infectious diseases. Milstein and Köhler's pathbreaking work on monoclonal antibodies earned them the Nobel Prize in Physiology or Medicine in 1984, ushering in the era of progress in biotechnology and immunotherapy [40], [41], [42].

4-Induced pluripotent stem cell (iPS) phenomenon

In 2006, Shinya Yamanaka and colleagues demonstrated that it is possible to reprogram adult mouse tail-end fibroblast cells into embryonic stem cells by simultaneous induction of four transcription factors (Oct3/4, Sox2, Klf4, and c-Myc). Reprogrammed cells were selected based on early development and the presence of Fbx15 gene expression, which is characteristic of embryonic stem cells. Induced pluripotent stem cells (iPSCs) exhibited characteristics of mouse embryonic stem cells (ES) but showed differences in gene expression and chromatin organization compared to ES cells [43][44]. The researchers then showed that selection for Nanog expression after transduction of four factors (Oct3/4, Sox2, Klf4, and c-Myc) resulted in a cell population more similar to ES cells. They injected the mouse retrovirus receptor into human cells to achieve a higher transduction frequency with amphotropic retrovirus. With this procedure, 60% of the cells exposed to the retrovirus expressed a reporter gene. Then, the same four genes were injected into adult human dermal fibroblasts, and the first human iPS cells were generated. Selection was based on the morphology and growth characteristics of these cells. It was also found that each iPS clone carried between three and six retroviral integrations for each of the

four factors. The most important feature of human iPSs was the capacity to form tissues of all three germ layers in tissue cultures and transplants [43]. B-4-Induced pluripotent stem cell (iPS) phenomenon. Shinya Yamanaka and colleagues had demonstrated in 2006 that adult mouse tail-end fibroblast cells can be converted into embryonic stem cells by the simultaneous activation of four transcription factors (Oct3/4, Sox2, Klf4, and c-Myc). Reprogrammed cells were enriched through early development and Fbx15 gene expression typical of embryonic stem cells. Induced pluripotent stem cells (iPSCs) had characteristics of mouse embryonic stem cells (ES) but were distinct from ES cells in gene expression and chromatin structure [43], [44], [45]. The authors went on to show that selection based on Nanog expression after transduction with four factors (Oct3/4, Sox2, Klf4, and c-Myc) resulted in a cell population more similar to ES cells. They transfected the mouse retrovirus receptor into human cells to achieve a higher frequency of transduction by amphotropic retrovirus. 60% of the retrovirus-treated cells expressed a reporter gene using this treatment. The same four genes were subsequently transfected into adult human dermal fibroblasts, and the first human iPS cells were generated. Selection was based on the growth and morphology of these cells. Also noted was that every iPS clone had between three and six retroviral integrations in each of the four factors. The most important feature of human iPSs was that they could give rise to tissues of all three germ layers in tissue cultures and transplants[43]. Chin et al. in 2009 reported a comparison of three human ESC lines versus five iPSC lines. The authors reported differences in gene expression in hundreds of genes [46]. Deng et al. [46]. Deng et al.[47] and Doi et al.[48] found variations in DNA methylation and told us that donor cells possess epigenetic memories in human iPSC. In 2012, the Nobel Prize in Physiology and Medicine went to John B. Guordon (for discoveries showing the reversible nature of cell specialization) and to Shinya Yamanaka (for reprogramming mature

mouse cells into immature cells). Both discoveries hold great importance in a broad spectrum of medicine disciplines such as oncology and regenerative medicine. ESC has also proven to be successfully used in cartilage repair, peripheral nerve, and cardiac regenerative therapy. MSCs have also been used in certain types of therapy, such as autologous transplants or the treatment of hematopoietic disease[49][50].

Cell culture uses

Cell culture is used in many fields. Here are some examples:

1. Drug and cosmetic product development: Cell culture is a preliminary research tool used in the development of drugs and cosmetics. The efficacy and safety of new drugs are determined by testing them on cells in the laboratory [51][52].

2. Cancer research: Cell culture is used to research the growth and behavior of cancer cells. The effect of drugs used in cancer treatment can be determined by testing on cell cultures[53].

3. Virus research: Research on the structure, growth, and effects of viruses is done on cell cultures. These studies are important for understanding how viruses replicate and spread [54][55].

4. Genetic research: Cell cultures are used to research the function of genetic material and how it works. In particular, cell cultures are important for developing and testing vectors used in gene therapy[56].

5. Biotechnology: Cell cultures are used in many fields in the biotechnology industry. For example, protein production, antibody production, and biofuel production[57], [58], [59].

6. Tissue engineering: Cell cultures are used in tissue engineering and transplantation studies. These studies are important for the

regeneration of damaged or diseased tissues and organs in the human body [60][61].

Cell Culture Techniques

A. Cell culture basics:

Cell culture is a technique by which living cells may be grown and sustained under controlled conditions in non-tissue media. Cell culture is widely applied in a wide range of fields such as medicine, biology, biotechnology, and pharmaceuticals. Cell culture basics involve the following steps: 1. Cell source: The cell source of the cell culture can be fresh cells of living organisms or tissue, frozen cell bank, or pre-cultured cell lines. 2. Culture medium preparation: A suitable medium is prepared in which the cells can grow. Culture medium should have nutrients, hormones, growth factors, vitamins, and minerals for the cells. pH, oxygen level, temperature, and humidity should also be checked. 3. Seeding the cells: Cells are seeded into the culture medium. During seeding, it is ensured that the cells have the correct density and are evenly distributed. 4. Provision to the culture: The culture medium is changed at regular intervals so that cells can grow and proliferate. Adequate nutrition is necessary for cell health and growth rate. 5. Examination of the culture: The culture is inspected with a microscope or other imaging techniques. Cell growth, shape, and activity are monitored. With various methods of manipulation, cell culture can be utilized for a wide range of research purposes, such as cell differentiation, cell-to-cell communication, cell behavior, and drug and toxicity screening.

B. Cell culture Materials and equipment

Materials and equipment required for cell culture can be listed as:

Materials:

Cell culture plate or petri dish: A sterile, flat-bottomed container to hold cells and culture medium. It has a wide surface area for cell attachment and growth.

Cell culture flask: Vented lid or filtered cap, sterile, round-bottom flask. It has a larger volume for cell culture and is used regularly to scale up cell cultures.

Cell culture media: Nutrient solutions containing essential nutrients, growth factors, and buffering agents to support cell growth. Different media formulations depend on the cell type.

Fetal calf serum (FBS): The most common supplement added to cell culture medium and derived from the blood of fetal cows. It provides essential growth factors, hormones, and proteins to stimulate cell growth.

Reagents and supplements: Various reagents such as antibiotics, growth factors, cytokines, and hormones can be added to cell culture medium to facilitate cell growth, differentiation or for particular experimental requirements

Equipment

Biosafety cabinet or laminar flow cabinet. Laminar flow cabinets have two types according to airflow: horizontal and vertical.

horizontally laminar airflow biosafety cabinets and vertically biosafety cabinets. The two types do the same thing in creating the correct sterile working area by removing particles that are a biosafety risk in the air using HEPA (high efficiency particulate) filters. The air is sterilized in horizontal cabinets because the air stream is blown straight into the researcher. It protects the environment and the product, but it has a drawback in terms of protecting the personnel. The air flow is blown vertically ahead of the personnel in vertical

biosafety cabinets, and the glass partition ahead of the cabinet shields the personnel.

The cabinets also vary in their biosafety classification, depending on the risk of biological contamination to the worker. Biosafety cabinets, or Class A biosafety cabinets, are robot systems for experiments with high biological contamination risk and are economically highly expensive. Class C laminar cabinets are purely product protection-oriented. These laminar flow cabinets are helpful equipment for realizing biosafety precautions. HEPA filters trap particles that may be a biosafety hazard in the workplace. In these cabinets, workers and researchers can work in good health and reduce the possibility of contamination to the same level.

Centrifuge: Centrifuge machinery is machinery that employs centripetal force to cause the cells to precipitate at the bottom of the reaction tubes using rotors that spin at a predetermined rate of rotation. The machinery consists of rotors suitable for the number of reaction tubes to be used. The rotors are usually designed for 1.5 mL, 15 mL, and 50 mL tubes and are available as replaceable or fixed rotors. If precipitation of cells alone is to be done, centrifugation at 800-1000 rpm for 1-2 minutes is sufficient. Centrifugation at higher speeds and for longer periods is not recommended to kill or destroy the cells. Additionally, if reaction tubes are to be mixed within a short period or the cap or cover material must be brought down to the bottom, the presence of handy mini centrifuges capable of centrifuging 0.5-2 mL reaction tubes saves time. Microscope: A useful piece of equipment for examining the morphology of the cells, determining growth, and checking the viability of the cells. Inverted microscopes are usually used for examining cell cultures, wherein cells are growing on the bottom of the culture dish**.**

Water bath Cell culture medium is stored in +4°C fridges and needs to be at 37°C before it interacts with cells. So, water baths are necessary. Other than that, cells stored in liquid nitrogen tanks are also thawed in water baths. So, the water baths need to be cleaned from time to time at regular intervals to prevent cell contamination. If work is interrupted for an extended period of time, the water bath needs to be drained.

Cryopreservation apparatus: Cryovials (cryogenic storage vials used to freeze and store cells long term with cryoprotective reagents and liquid nitrogen storage containers) are used to preserve the viability of cells for future use.

Cell Counting devices: Devices like hemacytometers, cell counters, or flow cytometers are machines that will accurately count cells and evaluate cell density.

Carbon dioxide incubator: laboratory incubators that are used for cell culture studies in which carbon dioxide (CO2) and humidity are controlled automatically. These incubators are built to mimic the natural conditions of the cells. Closed systems are usually carbon dioxide incubators and control the CO2 level of the atmosphere inside to the desired. Sensor systems are utilized for this purpose, and CO2 is continuously measured. CO2 gas is admitted into the incubator in a controlled form to achieve the desired concentration of CO2. Humidity is also controlled in carbon dioxide incubators. To this end, water is stored at the bottom of the incubator, and moisture is supplied through evaporation. The cells' need for moisture is hence satisfied, and optimum growth conditions are established. Carbon dioxide incubators control air circulation, too. The air inside is repeatedly circulated, resulting in an even condition. This ensures the CO2 and water of the media get distributed more evenly and enter the cells evenly. Due to these factors, CO2 incubators play a vital role in cell culture studies. In maintaining cells at the proper

temperature, CO2, and humidity, they enable researchers to have uniform outcomes. Autoclave: Used to sterilize equipment, solutions, and media using high-pressure steam, thus killing microorganisms.

Pipettes and pipette tips: Used for precise measurement and fluid transfer in cell culture. There are mechanical and electronic types.

Vacuum pumps are also used for the removal of PBS or nutrients after washing. Using a sterile glass pasteur pipette at the end of the pump tubing, culture liquids can be easily suctioned away. This is for the sterility cleaning of the liquids.

Microscope: In cell culture laboratories, two types of microscopes are used for the examination of cell viability and morphological features: an inverted microscope and a fluorescence microscope. The reason for the use of an inverted microscope is that cells must never be observed from the top of culture plates, and culture plates must be opened only in a laminar cabinet. This made it necessary to place the objectives at the bottom of the chamber. Fluorescence microscopes were developed with immunofluorescence staining techniques to view the protein structure of organelles in cells. Using these microscopes, fluorescent antibody-stained organelles become visible. Today's times have fluorescence attachments to inverted microscopes, such that two microscopes are utilized as one. This style enables one to see both the overall picture of cells and fluorescently stained organelles through the same microscope.

Refrigerators: The storage of the consumables used for cell culture is as follows:

FBS, trypsin, L-Glutamine, and antibiotics should be stored at -20 °C.

All the other culture buffers (DMEM, F12, RPMI, etc.) should be stored at +4 °C.

For cell freezing, they should be placed in styrofoam boxes and slowly frozen in -80 °C coolers a day before putting them in a liquid nitrogen tank.

The cell culture vessels are disposable, sterile, and polystyrene. 25, 75, and 125 cm^2 flasks are the most common flasks used for routine multiplication of cells. The flasks are divided into two types according to their lid type: ventilated and non-ventilated.

Flasks with ventilated closures. They are designed to allow easy exchange of CO_2 within the incubator. The filter on the closure is designed to prevent the entry of contamination agents such as fungi or bacteria. Air flow is thus ensured while at the same time reducing the possibilities of contamination.

Containers having lids without ventilation are used slightly ajar, not tightened completely, after being placed in the incubator. These containers allow for air to enter during the time the cells will be exposed to the environment. However, they should be used carefully to reduce the risk of contamination.

Cell culture plates of different sizes and well numbers are also used. Cells are generally seeded in 6, 12, 24, 96, or 384-well plates. They have a flat bottom surface and are made of polystyrene material. These plates are sterilized by gamma rays to remove DNase, RNase, and pyrogens. This provides a suitable condition for cell culture.

There are also insert plates that consist of two nested sections of chambers. The top section of these plates is where the cells are seeded and separated by a membrane whose pores have a width of 0.4 μm. The bottom section is where the culture medium is the only item inserted. These plates are generally suitable for such studies as cell growth, endothelial and epithelial cell differentiation, transport of drugs or chemicals across basolateral and apical cells, and

Transepithelial Electrical Resistance (TEER) studies, where the growth of epithelial cells is monitored.

This is just some of the general material and equipment utilized in cell culture. Some of the requirements will vary, specifically depending on the cell type and experimental requirements. Proper aseptic technique and compliance with proper procedures are essential to successful cell culture experiments.

C. Preparation of cell culture media

Cell culture media preparation steps are:

1. Preparation of the components: Cell culture media consist mainly of such components as amino acids, vitamins, minerals, hormones, growth factors, glucose, and serum. The components are determined according to the requirements of the to-be-prepared media.

2. Media preparation: While preparing the media, first, the right sterile laboratory working area should be used, and all equipment should be sterilized. Cell culture medium is prepared by adding it to a medium such as sterilized water or PBS (phosphate buffer salt). The pH of the medium should be checked and regulated with the right pH meter.

3. Media sterilization: Once the media is prepared, it is sterilized using sterilized filters. Sterilization may also be done by methods such as oxygen gas, ethylene oxide, or autoclave. Sterilization is of great importance to prevent contamination of cell culture media.

4. Storage: Once the media is sterilized, it is preserved by using sterilized bottles or containers. Media is usually preserved in the refrigerator or freezer.

5. Media handling: The media must be brought to the desired temperature in an appropriate incubator before cell culture media usage. The cells are inoculated into the media and grown under

appropriate conditions. The cells should be provided with appropriate conditions for proliferation and growth. The cells should be sub-cultured and the media replaced to maintain cell culture experiments.

D-Media Solutions in Cell Culture

Certain substances are needed in the cells' milieu in order to support cellular metabolic processes. These include amino acids, carbohydrates, vitamins, ions, growth factors, and hormones. Other factors such as temperature, humidity, carbon dioxide (CO2) and pH constitute the microenvironment required for cell growth. The medium composition on which cells are being cultured can vary depending on the type of cell, how suitable it is, and from what type of organism it has been obtained. To prepare the best composition of the medium, one has to refer to the literature in science. Basically, solutions to be added to the cell culture medium are listed below.

Dulbecco's Modified Eagle Media (DMEM)

Dulbecco's Modified Eagle's Medium (DMEM) is a very common cell culture medium that supplies the basic nutrients and growth factors needed for the growth and survival of many different types of mammalian cells in tissue culture.

The first to be developed by Eagle in the 1950s, Minimum Eagle's Medium (MEM) was established as the major source of amino acids and was eponymously named after Eagle. It was then modified by Dulbecco, and it became known as DMEM and continued to be the most frequent source of amino acids for monolayer cell cultures. DMEM can provide cells with a nutritionally supplemented environment.

Glucose is used as a source of energy, phenol red as an indicator of pH, and bicarbonate as an indicator of osmolarity. DMEM also contains vitamins and amino acids. Phenol red maintains the medium

pH at about 7.4 and is red, changing color to orange or yellow if the pH shifts in an acidic direction and to purple when the pH shifts in an alkaline direction. The color is seen when cellular catabolic wastes accumulate and indicates a need to dilute the cells or change the medium. Color shift can also occur in the case of bacterial or fungal contamination. L-glutamine is an unstable amino acid and a cell medium required component. Though L-glutamine is present in some DMEM solutions, because it has a short half-life, a stored medium for the long term will have no L-glutamine activity, and may be lost from the medium. In this case, 292 µg/mL L-glutamine should be added to the medium.

FBS (Fetal bovine serum)

Fetal calf serum (FCS) is among the most widely applied cell culture medium ingredients.

It is obtained from fetal cow blood drawn during meat processing for human consumption. FCS is a rich source of various growth factors, hormones, proteins, and other essential constituents. It has essential nutrients required for cell viability and optimal growth. FCS also includes binding factors that support cell adhesion. FBS is widely employed as an additive for the stimulation of cell growth, proliferation, and overall cell health in cell culture medium. FBS batches can vary in quality and composition, and therefore high quality FBS must be selected. Serum-free or defined formulae for culture media have also been formulated as substitutes to address some of the risks associated with the use of FBS. When serum-free media are used, attachment and growth factors can be added to the medium. Incubation will be used to denature material that otherwise makes FBS toxic to cells. 10% FBS is usually added to the cell culture medium. For hormone studies, DCC-treated FBS can be used in a manner where the hormones contained in FBS are not part of the result of the experiment. This is done by eliminating the hormones from FBS, and the combination of DCC-FBS is stored cold.

Antibiotic and antifungal solution

Antibiotic and antimycotic drugs are used in cell culture studies to prevent the risk of bacterial and/or fungal infection. The drugs are added to the medium in which the cells are grown. Commercial formulations are mostly employed and will normally consist of 100,000 U of penicillin, 10 mg of streptomycin, and 25 µg of Amphotericin B. The synergistic activity of this combination usually covers Gram-negative bacteria, Gram-positive bacteria, fungi, and yeasts. The growth of these microbes is inhibited by antibiotics and antimycotics, and reduces the risk of contamination. High-concentration antibiotics have also been reported to cause toxic effects, however. Consequently, in your laboratory, antibiotics are added to the media at 1% strength to reduce their potential toxicity. This is performed in an effort to minimize the risk of toxicity so that it can harmlessly influence the health of cells without disrupting their efficacy.

Phosphate Buffer Saline (PBS)

This salt solution contains the salts sodium hydrogen phosphate, sodium chloride, potassium chloride, and potassium dihydrogen phosphate. They form a non-toxic and isotonic solution to most cells. It stabilizes the osmotic pressure inside and outside the cell as well.

The structure of this solution is: 137 mmol/L sodium chloride (NaCl), 2.7 mmol/L potassium chloride (KCl), 10 mmol/L sodium hydrogen phosphate (Na2HPO4), and 1.8 mmol/L potassium dihydrogen phosphate (KH2PO4). It is most frequently used for cell washings in cell culture work. It may also be used as a solvent. It is

particularly important in maintaining an efficient osmotic environment for cells to grow and multiply in a healthy condition. The salt solution is an important material extensively applied in medicine and biological studies. In cell culture research and tissue engineering, it is beneficial in allowing cells to have a culture environment similar to that of the in vivo environment and in maintaining an equilibrium in osmotic pressure.

Tripsin

Tripsin bir tür serin proteaz enzimidir. Bir kimyasal olarak hücre kültürü deneylerinde kullanılır. Tripsin, lizin ve arginin gibi amino asitleri karboksil bölgede parçalamak suretiyle hücreleri birbirinden ayırır ve yapışık hücrelerin geçişini kolaylaştırır. Tripsin, hücrelerin birbirlerine yapışık olarak büyüdükleri substrata yapışmasını önleyerek hücrelerin kolayca ayrılmasını kolaylaştırır. Bu, pasajlama esnasında hücrelerin kültürden çıkarılması ve yeni bir kültür kabına aktarılması kolaylaşır. Tripsin stok çözeltisi çoğu zaman -20°C'de tutulur. Bazal sıcaklıkta depolama, enzimin stabilitesini ve aktivitesini sağlayarak korumasına yardımcı olur. Stok çalışma çözeltisi çözülür ve ihtiyaç duyulduğunda hücre kültürü araştırmalarında kullanılacak biçimde hazırlanır. Tripsin, hücre kültürü laboratuvarlarda sıklıkla kullanılan oldukça önemli bir kimyasaldır. Hücrelerin pasajlanması esnasında kullanıldığında, hücrelerin sağlıklı ve düzenli olarak çoğalmasını ve yeni kültür kabında çoğalmasını sağlar. Bu adımlar hücre kültürü deneylerinde ortamlık hazırlama temel adımları içermektedir.

C. Cell culture storage and cryopreservation

Cell culture can be stored in the lab for long-term storage. However, the ability of cells to grow and divide decreases with time, and cells can die and become inactive. Therefore, cell culture storage methods allow cells to be stored and thawed for reuse.

Cell culture storage conditions can vary depending on the cells, their use, and function. The majority of the forms of storage utilized involve the use of refrigerators (4°C) or freezers of lower temperatures (-80°C) for short-term storage. Cryopreservation, however, is more suitable for long-term storage and shipment.

Cryopreservation is the process of freezing and storing cells at very low temperatures, such as liquid nitrogen (-196°C). Cryopreservation stops the metabolism of the cells and preserves their ability for growth and reproduction. Cryopreservation is done using a special cryoprotectant solution. This cryoprotectant solution protects cells from freezing and thawing damage.

Cell cryopreservation is highly important to maintain the viability of cells and their activity. During freezing, the process has to be carried out very gently as there is a high possibility of cell contamination, cell loss, and death. In preparing cells for recycling, the cryoprotectant solution is thawed slowly, and cells are dissolved in a suitable nutrient solution. During thawing, the cells' viability and activity are checked very cautiously.

D. Best Practices for Maintaining Cell Culture Integrity

Cell culture integrity maintenance is of utmost relevance to reproducible experimental results and comprises meticulous compliance with Good Cell Culture Practice (GCCP), good documentation, and ethical concerns. Prevention of contamination is of utmost relevance and entails meticulous compliance with aseptic technique. All manipulations must be carried out in biosafety cabinets with HEPA filtration, and surfaces must be disinfected with 70% ethanol or 5% Trigene. Culture flasks should never be stored in non-sterile conditions, and overnight UV sterilization is recommended to

avoid maximum microbial contamination. Hand hygiene also enters the scene in a bid to avoid contaminant introduction. Hands are washed properly, gloves are ethanol-disinected, and clean laboratory-dedicated coats are donned. Sterile media and additives are always employed, but non-sterile additives must be filtered on 0.22 μm filters. Autoclavable, resistant, and high-heat equipment must be employed. Penicillin/streptomycin, the most avoided antibiotics, can be utilized, but should never be utilized whenever possible because they suppress the infection or cause antimicrobial resistance.

Cell culture must be observed carefully for early detection of contamination. The routine microscopic exam must be examined for abnormalities in morphology, turbidity, or color. Mycoplasma testing by PCR, ELISA, or fluorescence staining must be done regularly, as these kinds of contaminants can invalidate experiments. Contaminated cultures must be discarded at the earliest sign of contamination, and equipment must be decontaminated using bleach. Antifungals should be used cautiously to avoid spore release or cell toxicity. When working with human cell cultures, strict Biosafety Level 2 (BSL-2) or higher practices should be followed, and donor screening should be done to avoid hazards [62]. To avoid media-related problems, CO_2 is maintained at 5-10% or pH is buffered with HEPES. Nutrient depletion is prevented by log-phase subculturing, and batch-tested high-quality sera or serum-free medium reduce variability and contamination risk. To overcome adherence problems, trypsinization time may be reduced, or attachment factors may be supplemented, or biologically relevant substrates such as extracellular matrix coatings may be used to enhance cell adherence. Periodic cell line authentication through karyotyping, DNA fingerprinting, or Short Tandem Repeat (STR) profiling helps preserve the identity and origin of cell lines from genuine banks.[63]. Selective media or enzymatic digestion should be used to prevent fibroblast overgrowth in primary cultures. Senescence may be reduced by low-passage or immortalized cells, and stem cell differentiation, as well as genetic stability, may be

enhanced by newer approaches such as CRISPR-based editing and personalized scaffolds. Successful cell culture maintenance and reproducibility, as well as reliability, in research findings are due to rigorous quality control, cryopreserved backup stocks, and comprehensive GCCP training [64].

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BÖLÜM 3
Robot İşletim Sisteminde İnsan Davranışının Poz Tahmini ile Tespit Edilmesi

Ayla Eren Özdemir

1. Giriş

Otomatik yönlendirmeli araçlar [1] (OYA), endüstri, lojistik ve sağlık alanında giderek artan oranda kullanılan, çevreyi algılayabilen ve otonom hareket edebilen robotik sistemlerdir. İnsan yoğunluğunun yüksek olduğu acil servis, yoğun bakım veya fabrika zeminleri gibi alanlarda bu araçların güvenli şekilde çalışabilmesi için yalnızca engel algılama değil, insan davranışları [4]nı anlık olarak yorumlayabilen akıllı sistemlere ihtiyaç duyulmaktadır.

Bu çalışmanın temel amacı; Robot İşletim Sistemi (ROS) tabanlı bir ortamda, görüntü işleme yöntemleri ve derin öğrenme [11] modelleri kullanarak insan davranışları [4]nı gerçek zamanlı olarak tespit eden bir yapı geliştirmektir. Poz tahmini algoritmalarıyla elde edilen eklem koordinatları üzerinden yürüyüş, koşma, düşme, oturma ve tehlikeli görülen ani hareketlerin sınıflandırılması hedeflenmiştir.

Araştırma kapsamında RPLIDAR [8] tarafından elde edilen haritalama verileri ROS üzerinde işlenmiş, ardından acil servis bekleme alanını temsil eden sanal bir ortam Gazebo simülatöründe oluşturulmuştur. Davranış sınıflandırması için OpenPose [9] kullanılarak iskelet çıkarımı yapılmış, daha sonra matematiksel yöntemler ve derin sinir ağı modelleri birbiriyle karşılaştırılmıştır.

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2. Robot İşletim Sistemi (ROS) ve OYA Sistemleri

Robot İşletim Sistemi (ROS), robot bileşenlerinin haberleşmesini sağlayan, sensör verilerini yöneten ve farklı robotik algoritmaların uyum içinde çalışabilmesine imkân veren modüler bir yazılım platformudur. ROS'un sunduğu paket yapısı, veri akışı, düğüm iletişimi ve simülasyon desteği sayesinde robot sistemleri çok daha esnek ve genişletilebilir hâle gelmektedir.

Bu çalışmada kullanılan OYA sistemi;

• LIDAR ile çevresini tarayan,

• ROS-RViz ile ortamı gerçek zamanlı görselleştirebilen,

• ROS-Gazebo ile sanal bir acil servis ortamında test edilebilen,

• Görüntü tabanlı yapay zekâ modelleriyle insan davranışları [4]nı anında yorumlayabilen bir yapıda tasarlanmıştır.

OYA'ların özellikle sağlık ortamlarında güvenli çalışabilmesi, insan davranışları [4]nın doğru algılanmasına bağlıdır. Bu nedenle davranış tespit modülünün ROS ile entegrasyonu, robotun çevresel farkındalığını ileri seviyeye taşımaktadır.

3. Poz Tahmini ve Yapay Zekâ Yöntemleri

Bu bölümde davranış tespitine yönelik iki farklı yaklaşım geliştirilmiştir:

Poz Tahmini (OpenPose [9])

OpenPose [9] algoritması, insan vücudunun eklem noktalarını (el, kol, bacak, omuz, kalça vb.) yüksek doğrulukla çıkarabilen gelişmiş bir poz tahmini [4] yapısıdır. Çoklu kişi takibi yapabilmesi, gerçek zamanlı çalışabilmesi ve ROS ortamıyla uyumlu olması nedeniyle tercih edilmiştir.

Matematiksel Yöntem

Bu yöntemde OpenPose [9] tarafından elde edilen eklem noktaları arasındaki mesafeler, açı değişimleri, hız ve ivme değerleri ve zamana bağlı konum değişimleri hesaplanarak davranış sınıflandırması yapılmıştır. Temel davranışların tespitinde yeterli doğruluk elde edilmiş olsa da karmaşık hareketlerde performans düşmüştür.

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Derin Öğrenme Yöntemi

Derin öğrenme yaklaşımında 40 gönüllü katılımcıdan 7 davranış kategorisi toplanmış ve toplam 7000 görüntüden oluşan etiketli bir veri seti oluşturulmuştur. Eklem noktaları sinir ağı için giriş vektörüne dönüştürülmüş, model eğitimi ReLU aktivasyonu, Batch Normalization [12], Softmax çıkış katmanı ve çok sınıflı çapraz entropi kaybı kullanılarak gerçekleştirilmiştir. Model eğitim sonucunda %94 doğruluk elde edilmiş ve matematiksel yöntemden çok daha üstün performans göstermiştir.

Ortam Haritalama

RPLIDAR [8] 360° sensörü kullanılarak ortamın lazer taraması alınmış, veriler ROS üzerinde işlenmiş ve SLAM yöntemleriyle harita çıkarılmıştır.

Simülasyon Ortamı

ROS-Gazebo'da bir acil servis ortamı modellenmiş; ortam içinde insanlar, engeller, sedyeler, bekleme bölgesi ve hareketli objeler eklenmiştir.

Veri Seti Oluşturulması

Her katılımcıdan yürüme, koşma, düşme, bayılma, oturma, ayakta durma ve ani yön değiştirme gibi davranışlar için kayıt alınmış, görüntüler OpenPose [9] ile işlenerek eklem koordinatları çıkarılmıştır.

Sınıflandırma Algoritmaları

Matematiksel yöntem ve derin öğrenme [11] modelleri hem simülasyon ortamında hem de gerçek zamanlı testlerde çalıştırılmıştır.

5. Bulgular ve Tartışma

Matematiksel yöntemde davranış tespiti %70 doğruluk ile sınırlı kalmıştır. Özellikle karmaşık hareketlerde eklem açılarının benzerlik göstermesi hatalara yol açmıştır.

Derin sinir ağı modeli ise %94 doğruluk sağlamış; yüksek genelleme başarısı, dengesiz sınıflarda bile güçlü performans ve gerçek zamanlı ROS ortamında hızlı yanıt verme kapasitesi ile oldukça başarılı bir sonuç sunmuştur.

Gazebo test ortamında robot, bir kişinin düşme veya panik koşusu gibi kritik davranışları milisaniyeler içinde tespit etmiş ve güvenlik risklerini değerlendirebilmiştir.

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Bu bulgular, poz tahmini [4] tabanlı derin öğrenme [11] algoritmalarının insan davranışı analizinde oldukça güçlü olduğunu göstermektedir.

6. Sonuç

Bu çalışma, ROS tabanlı bir OYA sistemine insan davranışlarını algılayabilme yeteneği kazandırmak amacıyla poz tahmini [4] ve yapay zekâ modellerini bir araya getiren kapsamlı bir yaklaşım sunmuştur.

Elde edilen sonuçlar şunlardır:

• Derin öğrenme modeli, insan davranışları [4]nı yüksek doğrulukla ayırt edebilmiştir.

• Matematiksel yöntemler temel hareketlerde işe yarasa da karmaşık davranışlarda yetersiz kalmıştır.

• Simülasyonun ROS ile entegrasyonu, gerçek uygulamalara geçiş için güçlü bir altyapı sağlamıştır.

• Sistem, acil servis gibi kritik ortamlarda güvenliği artırma potansiyeline sahiptir.

Gelecek çalışmalarda modelin gerçek ortam verileriyle yeniden eğitilmesi, davranış kategorilerinin artırılması ve robot kontrol mekanizmasına tam entegrasyon önerilmektedir.

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GEÇİCİ KAPAK

Kapak tasarımı
devam ediyor.