ROLE OF BIOREACTORS IN TISSUE ENGINEERING TOWARD CLINICAL ASPECTS

Abstract

Tissue engineering needs bioreactor technology the biological, biochemical, and biomechanical needs can be met in a controlled environment provided by a bioreactor to manufacture designed products. To provide an in vitro physiological environment that is particular to a certain tissue during tissue maturation, bioreactors are typically used. The intricate process of growing cells under controlled circumstances is known as cell and tissue culture. These cells are often of mammalian or plant origin. The right bioreactor must be chosen for the best growth of plant cell and tissue cultures to produce useful chemical components. The creation of recombinant therapeutic proteins by mass culturing of animal cells takes place in bioreactors, which are crucial in the field of biologics. Bioreactors can be created using designs for stirred tanks, airlifts, hollow fibers, or Rotary Cell Culture Systems (RCCS), among other configurations. The stirred-tank bioreactor is among the most well-liked designs, which is employed in both industrial and lab research. By definition, using bioreactors to study normal and pathology must be extremely different, and the physiological environment has an impact on how such bioreactors are designed. By providing circumstances that mirror the native milieu of the 3D tissues, bioreactors provide a great platform for growing and developing these tissues. High-value metabolite and therapeutic protein biotechnological production using It has been promoted that plant in vitro systems are an appealing replacement for conventional technologies.

Keywords: Bioreactor, Tissue engineering, Drug Discovery, Plant and Animal Cell, clinics.

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I. INTRODUCTION

In biotechnology, many bioreactors are employed depending on the bioprocess kinetics, hydrodynamics, and scale of operation. In a bioreactor, controlled process conditions are guaranteed while the creature responsible for producing the desired good does so. [1]. The requirement for Cross-disciplinary cooperation in the biochemical process business will show up more and more in advancements in bioreactor design, such as whole-cell immobilization, immobilized enzymes, continuous reaction, and process control[2]. Typically, a bioreactor provides an environment that controls the flow of nutrients, oxygen, and metabolic products to and from the cells through a biomechanical and biochemical process [3]. Because Bioreactors, unlike conventional chemical reactors, sustain and control living organisms. Bioreactor systems need to be strong enough to provide a greater level of control over process upsets and contaminations because organisms are more fragile and unstable than chemicals. [4]. Ideally, the bioreactor has suitable conditions for the demonstration of the activity of living microorganisms under regulated conditions. This requires certain special elements to be included in reaction engineering for biocatalytic processes [5]. A fermenter is a form of bioreactor that uses a living cell as the biocatalyst and is frequently referred to as a "bioreactor" in the scientific community.

Under aerobic or anaerobic conditions, the term "fermentation" refers to the development of microbes in food [6]. Under the right physiological conditions, these decellularized tissues are being recellularized in bioreactors [7]. Biological replacements are created through the process of tissue engineering to maintain, improve, or restore tissue function using engineering and life science ideas and techniques [8]. The goal of tissue engineering is to create an environment in vitro that closely resembles the biochemical and mechanical cues that regulate tissue growth and maintenance in vivo. An in vitro tissue engineering system is composed of three main components: metabolically active cells that can express their differentiated phenotype, polymeric scaffolds that provide a three-dimensional (3D) structure for cell attachment and tissue growth, and bioreactor culture vessels that provide an in vitro environment in which cell-polymer constructs can develop into functional tissues. [9]. numerous operational factors, such as Cell perfusion, pH, temperature, oxygen tension, and external stimuli such as mechanical forces, etc., can be changed and controlled [10]. If the bioreactors are to be used again, they must be able to be sterilized. In addition, sensors must be integrated for accurate culturing conditions monitoring, into them [11].

II. BIOTECHNOLOGY MILESTONE ON BIOREACTORS DESIGN

Since ancient times, the bioreactor has been a known historical device. Old antique clusters were able to overcome bioengineering design challenges for practical purposes like the manufacture of wine and beer with merely experience and observations. The creation of biotechnological processes was made possible as a result. Particularly those used in the preparation and manufacture of food products [12]. Scientists like Along with others, Lorenz Oken (1779–1851) and Theodor Schwann (1810–1882) started to understand the underlying concepts governing the actions of cells in the body and culture in the early nineteenth century [13]. These findings were expanded upon by Louis Pasteur (1822–1895) into a cogent explanation of the mechanics underlying fermentation [14]. Large-scale fermentation systems were established around the start of the 20th century, which affected the wartime industry at

the time. It was established to produce glycerol from yeast using glucose as a starting material for the explosives industry. Another recent illustration of this is the large-scale manufacture of butanol and acetone by butyric acid bacteria, as discovered by Chaim Weizmann and used first for explosives and subsequently for the manufacturing of rubber in the expanding auto industry [15]. An efficient design of a bioreactor should take into account increased productivity and validate the necessary parameters to produce goods that are dependable and of excellent quality. A bioreactor's design and operation are determined by the organism being produced, the ideal conditions needed for the desired product to form, the intended product's value, and the volume of production. A bioreactor's attributes should adhere to design specifications, such as sterilization and ease of building and measurement, process control devices, regulating approaches, scale-up, flexibility in operations, compatibility with upstream and downstream processes, antifoaming measures, etc. These are critical elements [3]. The headspace volume, the agitator system, the oxygen supply system, The essential components of a bioreactor are the foam control, the temperature and pH control system, the sample ports, the cleaning and sterilizing system, and the lines for charging and emptying the reactor.[16].

III. BIOREACTOR DESIGN

The design of a suitable bioreactor to develop goods that are consistent and of superior quality should take into account greater productivity and the cost-effective validation of desired parameters. [17]. Reducing production costs while still delivering high-quality goods or services is the main goal when designing an apparatus used in biotechnological processes, such as a bioreactor. The bioreactor is essential to the economics of biotechnological processes as it acts as a conduit between the raw materials and the finished output [2]. Evaluating the societal application of the system and its planned culture is one of the main priorities when constructing a bioreactor. To more fully comprehend the effects of various factors, such as fluid flow region, oxygen tensions, mechanical force stimulation, and their effects on cellular processes behavior, After conceiving the bioreactor design, prediction methods can be used to confirm the bioreactor's efficacy in carrying out the desired responsibilities [18]. To enable tissue growth on cellular 3D scaffolds under specific flow and/or mechanical conditions, it is important to precisely characterize and simulate bioreactor systems conditions [19]. Direct sparging, indirect and/or membrane aeration, medium perfusion, rising oxygen partial pressure, and rising atmospheric pressure are all examples of aeration techniques are all ways to aerate a culture. Any one or combination of these techniques may also be used [20].

IV. TYPES OF BIOREACTOR

Based on the procedure utilized to introduce the culture and media into the bioreactor. Here are a few other varieties that are described: photobioreactor, fluidized bed bioreactor, continuous stirred tank bioreactor, bubble column bioreactor, and airlift bioreactor.

1. **Airlift Bioreactor:** A gaseous stream is injected into pneumatically agitated bioreactors to facilitate mixing. Typically, this stream is air and simplifies the transfer of gaseous compounds (such as O2 and CO2) with the liquid phase. In contrast to conventional pneumatically agitated reactors, which randomly mix the liquid (i.e., bubble column),

airlift reactors (ALRs) have a unique design that allows the liquid to move between two connected zones known as the riser and the downcomer [21].ALRs can be set up in either one of two fundamental ways: external loop reactors or internal loop reactors. Unlike the second, which just has a strategically placed barrier within a single vessel that creates a barrier, the first one has distinct channels for fluid circulation. A central and a peripheric channel are created by a few channels for the tubes in a circle are be circulated [22]. Instead, a split baffle or a focused draft tube physically separates the riser and downcomer inside the internal loop ALR vessel. It may be possible to use either the annulus, depending on where the gas sparger is positioned or the concentric draft tube as a rise in internal-loop reactors[23].In terms of reactor design flexibility, external-loop systems are less flexible and compared to internal-loop ALRs, have so far supported a far smaller number of applications. For external ALRs, a control valve can be installed in the duct that connects the riser and downcomer, but Internal-loop ALR liquid circulation velocity mostly depends on gas input. Compared to internal-loop ALRs, external-loop ALRs typically offer higher liquid circulation velocities and slower mass transfer rates than split and draft tube ALRs [24].

2. **Two-Stage Airlift Bioreactors:** Regarding the production of goods that are influenced by temperature, two-stage airlift bioreactors are employed. Pumps are used to transfer growing cells from one bioreactor, which is kept at a temperature of 30°C, to another, which is kept at a temperature of 42°C. Because it is extremely difficult to increase the temperature from 30°C to 42°C in the same vessel, the two-stage airlift bioreactor is necessary. Each bioreactor has a valve, and a transfer tube and pump connect them. In the first bioreactor, the cells are cultivated, while in the second reactor, the actual bioprocess occurs.

3. **Tower Bioreactors:** An enormous pressure-cycle is fermented in a tower bioreactor. A strong hydrostatic pressure generated at the reactor's bottom increases O2's solubility in the medium. Reduces pressure and makes CO_2 ejection easier at the riser's top (with extended top). The cycle is finished when the medium returns to the downcomer. Tower bioreactors have the benefit of having high aeration capabilities without the need for moving elements. [21]
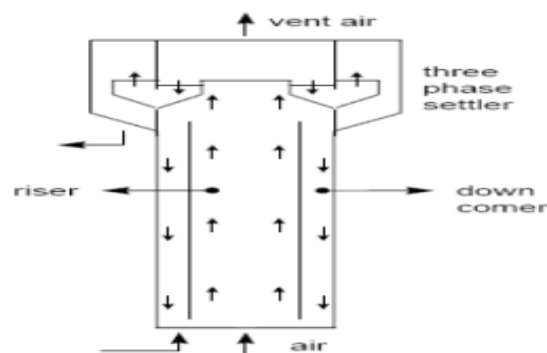

Figure 1: Tower Bioreactor [100]

4. **Bubble Column Bioreactor:** A cylindrical vessel with a bottom-mounted gas distributor and a bubble column reactor (BCR) is used to create bubbles. A liquid phase or a suspension of a liquid and solid is created from the gas by sparging it with bubbles. These

reactors are frequently referred to as slurry bubble column reactors since they have a solid phase. The industries of metallurgy, petrochemistry, biochemistry, and chemicals heavily rely on bubble columns as multiphase contactors and reactors [25, 26]. High catalyst durability or other packaging material, online catalyst addition, and catalyst removal capability make bubble column reactors the favored choice [27]. The basic design parameter known as the gas holdup explains the transport properties of bubble column systems and is dimensionless. The proportion of the gas phase's volume that is made up of gas bubbles is the primary definition. Because every research must include a topic on the design and analysis of bubble columns and gas holdups. Despite the ease with which bubble columns can be constructed, good design and scale-up require a deeper understanding of multiphase fluid dynamics and its influences [26]. A better knowledge of multiphase fluid dynamics and its effects is necessary for efficient design and scale-up, notwithstanding the ease with which bubble columns can be built. Their design is based on the reacting system's kinetics of chemical reactions, heat, mass transport, and mixing characteristics [28].
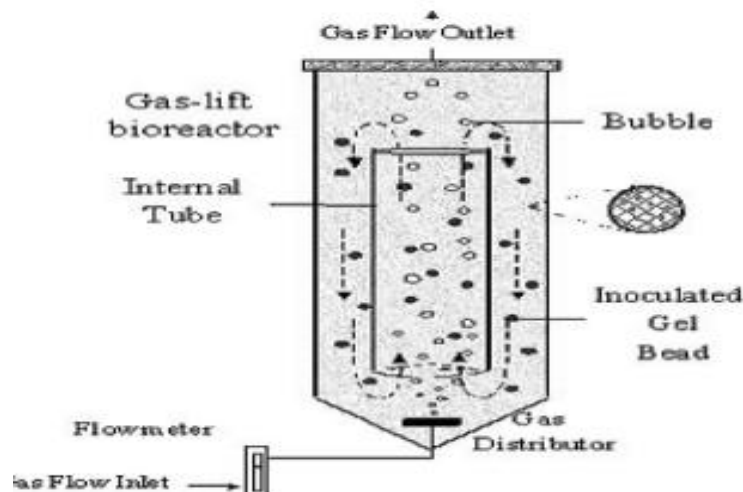

Figure 2: Bubble Column Bioreactor [100].

5. **Continuous Stirred Tank Bioreactor:** For a variety of reasons, the most popular anaerobic reactor (AD) runs in steady-state and is known as a completely mixed anaerobic digester or continuous-stirred tank reactor. Using continuous agitation, or CSTR, bacteria are continually dissolved with the components of the effluent [29]. By sparging the mixture, constant mixing is made possible, and it also aids in the removal of harmful effluents from wastewater [30]. The term "Solid retention time" (SRT) is the period wastewater solids are held in an anaerobic digester as opposed to "hydraulic retention time," which is the period a substrate is held in a digester. [31]. HRT and SRT are equivalent in the case of CSTRs.As opposed to those that have a bigger HRT than SRT, this indicates that the conversion rate of effluents into biogas occurs at a slower pace and produces the lowest output [32]. For Higher rates should be avoided. A reasonable quantity of CH4 generation should occur in CSTR, an HRT/SRT should have a range of 10-15 days, and the OLR should be less than 8 g VS/L d. Numerous anaerobic reactors were chosen and added to produce enough biogas; the best HRT for that range is intermittent mixing with a medium intensity. [33].
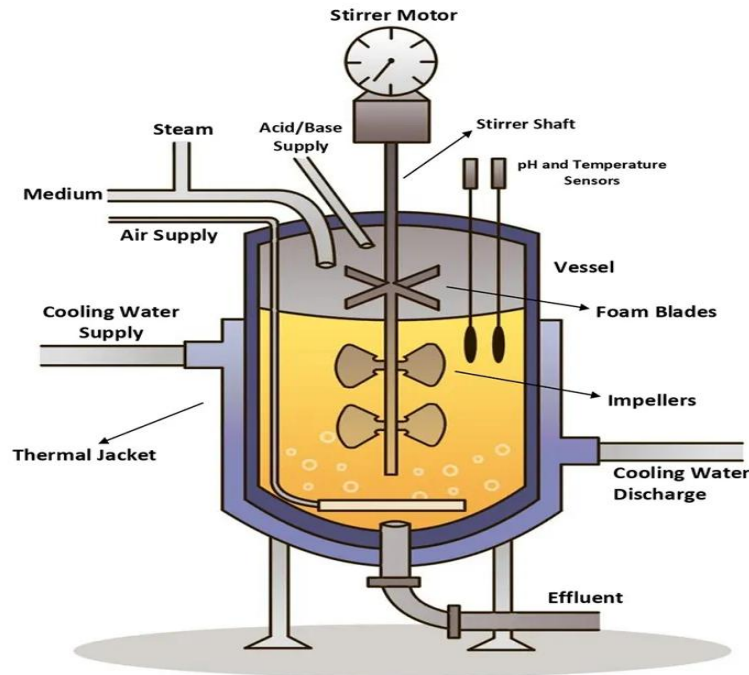
Figure 3: Continuous Stirred Tank Bioreactor

6. **Fluidized Bed Bioreactor:** Fluidized bed reactors (FBRs) are packed beds of lower particle size. These reactors contain minute particles that move with the fluid and immobilize the cells. The supply of mass, oxygen, and nutrients to the cells can all occur at a faster rate because of smaller particles. Washout-related restrictions on free cell systems can be overcome and the biocatalyst concentration can be significantly increased [34]. The adhesion of suspended particles to the aid that will be treated when the flow rate is increased is what determines the efficacy of a fluidized bed reactor. The particles, which are frequently referred to as "biofilm carriers," can either be porous particles that trap the biocatalyst or inert cores on which the biomass is formed by cell adhesion.

The Co-current up-flow is used to operate hydrated reactors with liquid as the continuous phase. Particle entrapment, internal component degradation, pressure loss, higher pumping requirements, and pressure drop are just a few of the unfavorable characteristics of FBRs [35]. Tall columns that have a generally greater than 10:1 height-to-diameter (aspect ratio) ratio are fermenters used in fluidized bed (FB) operations. Fermenters that have been designed and operated have an aspect ratio of either 20:1 or 40:1. The optimal ratio for the column diameter to particle diameter is at least 50:1, although, in the laboratory, it is frequently necessary to make concessions to keep the fermented volume as small as possible [36]
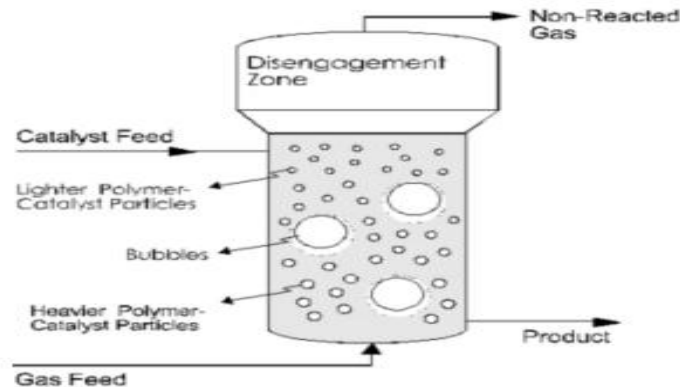
Figure 4: Fluidized Bed Bioreactor [101]

7. **Packed Bed Bioreactor:** The Packed Bed Bioreactors (PBRs) are made up of a packed bed that supports the cells on or inside carriers and a reservoir used to recirculate the oxygenated nutritional medium through the bed. The packed-bed and other designs comprise the two primary types, inside and outside of the medium reservoir [37, 38]. An immobilized biocatalyst is placed inside the column, which is subsequently fueled with nutrients from either the top or bottom. Through the solid bed, a fluid that contains dissolved substrate and nutrients flows. Substrate contact with the bed can be modified by varying urine flow rate and residence time. Either within or outside of the medium reservoir is where the packed-bed compartment is located. To remove the external mass transfer restriction in the neighboring liquid hem, the channel flow speeds may be high. While plugging can be avoided concurrently, significant pressure loss results from doing so. [39].A matrix that offers the necessary ideal state is stated to combine cell attachment, proliferation, and productivity. Next, this matrix is utilized to improve the operating parameters of the PBR, such as packed-bed height and volume, medium perfusion rate, etc., utilizing perfusion studies normally performed at laboratory size. [40]
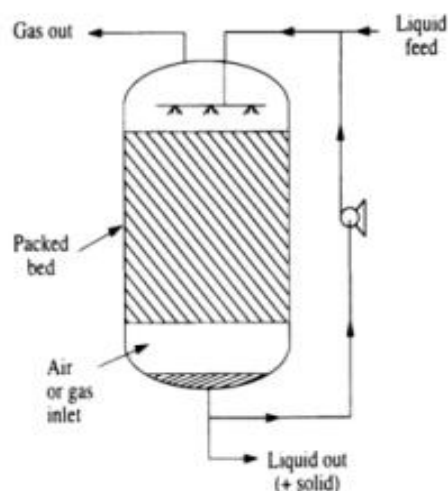

Figure 5: Packed Bed Bioreactor [21]

8. **Photo Bioreactor:** The development of microalgae or vegetable biomass under regulated conditions is one of the main applications of photobioreactors in photosynthetic processes. There are three types of photobioreactors: flat-plate, horizontal/serpentine tubular airlift, and inclined tubular, closed photobioreactor, open ponds, laboratory to industrial size models, and photobioreactors [41]. Photo bioreactors would have ideal substrate mixing with minimal In the case of photobioreactors, each microalgae cell would have access to the best light absorption in any place inside the bioreactor due to the absence of dead volume, hydrodynamic shear stress for the cells, and adequate gas-liquid mass transfer for CO_2 absorption and O_2 release. [42]. Ideal photobioreactor conditions are impossible to attain in practice, hence a variety of closed photobioreactor designs are available, each using a different strategy to promote the best possible production and expansion throughout the procedure [43].In contrast to low light intensities, which do not offer adequate energy for optimal growth, high light intensities produce photoinhibition of the algal light-harvesting system [44]. While industrial-scale photo bioreactors need artificial light, the sun frequently lights lab-scale photobioreactors. For growing on a laboratory scale, white, red, and blue light has been adequate, enabling the production of biomass or product concentrations with higher levels [45]. Processes for scaling up photobioreactors are more complex than those for traditional reactors since different types of light are available at different times [46]. Phototrophic microorganisms can already be grown on a big scale thanks to technology. It is more important to be practical on this size, which is dependent in large part on selecting the appropriate state variables and transferring technology to an industrial scale [47].

V. BIOREACTOR IN TISSUE ENGINEERING

Bioreactors for TE applications should, in general, make it easier to cultivate 3D artificial tissue under sterile circumstances and should be simple to use, such as for cleaning or simple construction. When switching from 2D cell culture technology to 3D tissue, it is also crucial to maintain mass transfer and manage the process parameters created constructions [48, 49]. The following categories can be used to classify basic and well-proven bioreactor designs that promote enhanced mass transport: Direct perfusion systems, hollow-fiber gadgets, spinning flasks, and rotating-wall containers [50, 51]. An ever-increasing variety of natural and recombinant microbial systems, immobilized and tissue-engineered enzymes, cells generated from animal tissues, and bioreactors are all used in biotechnology [83].

1. **Rotating Wall Vessels:** In rotating-wall containers, the scaffold is rotated continuously to create a constant circulation flow around the 3D cell constructs as they grow [52]. Although shear stress is crucial for modifying the mechanical characteristics of tissue constructs, excessive shear stress leads to the creation of undesirable capsules around the tissues, hence several bioreactors with minimal shear stress have been devised [53]. The rotating wall perused vessel (RWV) was first introduced by NASA, and it has since evolved into several derivative systems, such as the rotating wall perused vessel systems, the high aspect ratio vessel (HARV), and the slow lateral turning vessel (SLTV). The STLV is already available and is intended to fill the annular space between two cylinders (Synthecon, Houston, Texas). That are concentric and have a silicone gas exchange membrane inside of them. In contrast to the STLV, the HARV has a gas exchange

membrane at its base. These vessels are rotated Can contain up to 12 tissue constructions and 100-110 ml of culture fluid and rotate horizontally by solid-body rotation at rates of 15 to 40 rpm [54]. The RWV has also been modified to include a medium inlet at one end and a medium outflow via a filter on the middle cylinder (RWPV) to produce cartilage in space's microgravity environment [55]. Due to a rotation of the inner and outer cylinders, and the dynamic equilibrium of the active centrifugal, gravitational, and drag forces, creates microgravity and places the cell/scaffold structures in a state of free fall. Laminar flow has been observed to occur at the RWPV's surface constructs, and The maximum shear stress is in the range of 0.8 dyne/cm2 when a 120 ml RWPV with a single model build is operated with the inner and outer cylinders rotating at 13 and 37 rpm, respectively [56].

2. **Spinner Flasks:** Spinner flasks offer comparable culture conditions to those seen in convective fluid flow. Contrary to rotating-wall vessels, a stirrer mechanism creates convection in the medium of a spinner flask. The culture parameters are well evidenced by the fact that spinner-flask technology has been used for more than 15 years. [57]. Growing cardiac and cartilage constructs in spinner flasks lead to designed tissues that are superior to those grown under static conditions because they give the cells a well-mixed environment and decrease the stationary cell layer at the construct surface[58]. However, spinner The turbulent flow and increased shear stress that comes from using flasks may not be desirable because they promote the growth of cartilaginous tissue in conventional spinner flasks to produce an exterior fibrous capsule [53].

3. **Perfusion System:** The expected uniform 3D-cell coverage was not achieved due to inadequate cell infiltration into the core of the scaffold under static culture conditions, which resulted in the exterior of the scaffold being surrounded by a shell of cells[59,60]. Medium flow through the scaffold porosity stimulates cell differentiation by increasing nutrition delivery to the interior of the scaffold and mechanical stimulation from liquid shear [61]. TransCyte, a pair of tissue-engineered products, and Derma graft were produced using such perfusion bioreactors[62].To grow tissue-engineered bone utilizing stem cells from bone marrow, a second perfusion-chamber bioreactor has been used[63]. When cultivating cartilage, with cyclic hydrostatic pressure (0–5 MPa) applied at 0.5–0.3 Hz, the perfusion system can be employed in conjunction with intermittent hydrostatic pressure [64]. Another successful application of one perfusion reactor is the development of Human bone marrow stromal cells, a dematerialized bone matrix, and two layers of a vascular matrix were used to create an osteon-like tissue structure with improved tissue strength [65]. Dynamic seeding has also been shown to outperform static seeding in terms of effectiveness and uniformity sowing [66]

4. **Pulsatile Flow Reactor:** Four reactors, each containing a construct, are supplied with intraluminal pulsatile flow by the perfusion system. The pump's pressure is 27030 mmHg, and its variable stroke volume ranges from 0 to 10 ml/stroke. Pulse rates between 60 and 165 beats per minute can be used to operate the bioreactor. Because cardiovascular disease is one of the leading causes of morbidity and mortality worldwide, strong financial and medical incentives exist to create new blood arteries and myocardial tissues. Pulsatile perfusion vascular cells are subjected to pulsatile physical stimuli; hence bioreactors have been created to mimic the cardiovascular conditions in vitro stresses throughout much of vasculogenesis and life [67]. After being seeded onto biodegradable

polymeric scaffolds, cells of the endothelium and vascular fibroblasts are grown under circumstances that gradually increase the flow and pressure of the nutritional medium. SMC is also cultured on porous tubular scaffolds using a pulsatile flow method [68].

VI. BIOREACTOR IN DRUG DISCOVERY

Creating three-dimensional, functionally appropriate tissues in bioreactors now involves more than just that. Beyond regulating tissue growth and development, research has begun examining to eventually replace or do away with the use of animal models, bioreactors as effective in vitro testing platforms for pharmaceuticals and implants have been developed. For Understanding human physiology and disease biology, medication testing, implant testing, and using pre-clinical animal models are necessary. It is necessary to develop alternate methods for using animal models to advance Replacement, reduction, and refinement are known as the three Rs and create a foundation for compassionate animal research [69]. Due to the increased need for these 3D models, particularly 3D illness and cancer it has become essential to import tissue engineering technologies such as dynamic bioreactor systems[70]. As an alternative to using animal models to evaluate pharmacological drugs, some studies have shown how to use bioreactors to generate 3D tissues.

To better understand the biology of multiple myeloma and how it responds to treatment with medications, myeloma tissues were grown in the commercially available RCCSTM bioreactor with controlled maintenance of cells, architecture, and microenvironment. [71]. comparatively, to standard 2D monocultures, It was possible to maintain functional cells over an extended period by co-culturing hepatocytes and fibroblasts in a bioreactor under dynamic conditions [72]. Development of the rotating wall vessel bioreactor was used to create 3D hepatic-like tissues for drug testing with the aid of bile duct-like structures and blood vessels that resembled fetal liver cells. 3D cancer tissues that were perfused and grown in bioreactors had characteristics and reacted to drugs similarly to in vivo malignancies [73]. To construct ex vivo disease models using tissues in addition to drug testing, bioreactor platforms can be used from humans or animals to comprehend the molecular processes behind physiological states and disease progression [18]. Using perfusion bioreactors to replicate the fluid dynamics that describe the arterial/venous interface, it has been possible to analyze the arterializations of veins after artery bypass grafting characteristics[74,75].In a different investigation, Using a shear stress bioreactor allowed for analysis of the development of calcify valve disease and offered regulated hemodynamics to pig aortic valves[76].

VII. PLANT CELL USING BIOREACTOR

Process development and optimization heavily rely on the bioreactor. The most basic design is an uninstrumented cultivation vessel (such as a plate, tube, or flask) that is shaken or operated in an incubator. External machinery is to blame for the monitoring and management of crucial cultivation variables including temperature and shaker speed. These systems are on a modest scale and are well known from screening investigations (culture medium, cell line). Greater scale culture is done in instrumented bags and vessels, and the bioreactors have their own measuring and control equipment. This often means that the following parameters are tracked and managed online: temperature, agitation speed, aeration rate, and dissolved oxygen (DO). Online foam, pressure, filling level, pH, and conductivity

sensors are also available in plant cell bioreactors. By increasing cell density in the culture and lengthening the exponential active cell growth phase for the growth-associated foreign protein production (secreted or intracellular product) governed by a constitutive promoter, target protein productivity can be increased.

When employing a successful substrate-feeding approach, To achieve a high cell density, fed-batch cultures have been used. [78]. However, when the PCV reaches between 60 and 70%, the pace of cell expansion in fed-batch or batch plant cell culture is frequently slowed, which lowers the rate of cellular metabolic activity [78]. Accordingly, Due to plant cell aggregation, surface adhesion, and the high apparent viscosity seen at high biomass concentration, semi-continuous culture or perfusion culture with a bleed stream has been proven to be more appropriate for high cell density culture. It has been demonstrated that semi-continuous culture or perfusion culture with a bleed stream is superior to fed-batch and perfusion culture for increasing cell density is more effective for this purpose. For the manufacture of valuable natural goods including medications, flavors and fragrances, and fine chemicals, plant cell cultures have become increasingly popular. The commercial use of Nevertheless, research on plant cell cultures has been constrained by several issues. The inability to maintain photoautotrophic growth, slow growth, genetic instability, and low and irregular cell productivity are the main problems in development [79]. A bioreactor's design must also take into consideration particular qualities of plant cell cultures, such as their Large cell size, complex shape, aggregation tendency, time-dependent rheological behavior, foaming and wall growth, shear sensitivity, and comparatively modest growth and oxygen intake rates [80].

1. **Recent Advances in Bioreactors for Suspended Plant Cells**

- **Bioreactor hardware:** A stirred-tank unit is the most widely used type of bioreactor because it is easy to scale up, has good fluid mixing and oxygen transfer capabilities, offers several impeller options, and is easy to comply with current strong Manufacturing Practices (cGMP) rules. However, these bioreactors do have several disadvantages, such as high power needs, significant shear, and problems with shaft stability and sealing in tall bioreactors. As opposed to microbial cells, plant cells are more susceptible to shear, so it is necessary to make significant modifications and optimizations to the impeller system must balance the needs for mass transmission and mixing with the danger of hydrodynamic force damage. Designing new impellers has enabled numerous improvements to be made to traditional STRs [81]. A pneumatically agitated bioreactor, also referred to as an air-lift or bubble column bioreactor, is a type of gas-liquid dispersion reactor that consists of a cylindrical vessel into which compressed air or a gas mixture is typically introduced at the bottom for fluid circulation, aeration, and mixing without the need for moving mechanical parts. Compared to STR, this type of bioreactor provides the following benefits: characterized by more uniform energy dissipation [82].

VIII. ANIMAL CELL USING BIOREACTOR

Animal cells are currently finding practical use in the creation of proteins and their derivatives for medicinal, prophylactic, and diagnostic purposes. It is necessary to supply the bioreactor system with controlled amounts of feedstocks or substrate. The bioreactor must

then be able to retain its integrity throughout the production run and must synthesize the required product, which necessitates that the bioreactors carry the necessary information, including signals and data that support product creation. Consideration of the grown animal cell as a bioreactor can be made using these fundamental factors. As soon as the bioreactor is fully populated with cells, the cells are fed a new medium—possibly one with a different composition—to keep them healthy and ready to produce products.[83]. A variety of different and frequently desirable processes is used by animal host cells to process and change their expressed proteins. The finished product is biologically active, as opposed to recombinant proteins created by bacterial cells, which often take the form of an insoluble inclusion body that must be refolded into its normal conformation. [84, 85].

A significant part of the biologics manufacturing process currently involves animal cell cultivation in bioreactors [86, 87] Diagnostic monoclonal antibodies, protein medicines, and viral vaccinations are among the biological products made using animal cell culture. Aside from that, bioreactors have been used to artificially manufacture stem cells and other types of cells that are scarce [88, 89]. Animal cells, in contrast to microbial, plant, or other eukaryotic cells, lack a robust cell wall and have fragile membranes. Due to their relative size and slow growth, they are easily injured when grown in an artificial environment [90]. High-density animal cell culture ($>1x10e7/ml$), which reduces costs and increases output, is particularly alluring. Numerous variables, such as pH, temperature, dissolved oxygen, nutrient intake, metabolite accumulation, serum, growth hormones, extracellular matrix, and shear stress, have an impact on animal cell proliferation. [91]. Nutrient consumption and the buildup of metabolic waste are both associated with cell growth. To promote homogenous cell growth and ensure product quality, rigorous parameter selection is necessary [92].

IX. BIOREACTOR IN CLINICS

Given years of research successes in the bioreactor creation of transformed tissues, commercial and clinical usage of these systems is unquestionably near. To make successful vascular grafts with well-formed collagenous extracellular matrix (ECM), a bioreactor has been used to impart cyclic radial strain to cells growing on biodegradable scaffolds. the development of therapeutically valuable decellularized, tissue-engineered vascular grafts that can either be stored for future use or implanted after being seeded with autologous cells came next, and it used detergent-based decellularization.[93,94]NeoCart, a commercial product now undergoing clinical testing, makes use of a bioreactor to create circumstances that closely mirror the low oxygen tension and pressure conditions present in the knee[95,96]. Cytograft Tissue Engineering [97] is a different company that uses cell sheet tissue engineering to make vascular grafts and has a patented bioreactor technique. The bioreactor offers a mandrel and a cell sheet growth module for creating the intended blood vessel. Additionally, it has been shown that the company's Life Line graft is clinically effective [98, 99]. Clinical use of modified tissue produced in bioreactors is subject to several conditions, as seen from the clinician's perspective. We require tissue culture systems that are simple to use, secure, reproducible, automated, scalable, flexible, and consistent with regulatory requirements. These systems must also be capable of quickly controlling and evaluating the developing tissue and its microenvironment. Establishing the ideal culture conditions for tissue growth comes after choosing or making geometrically specified scaffolds, isolating particular cell types, and producing 3D tissues.

X. CONCLUSION

Over many years, bioreactors create a wide range of therapeutic biomolecules and other high-value goods. They provide the opportunity to continuously monitor and control environmental factors during the culture/reaction phase, in addition to the added benefit of maintaining a closed system. They are a necessary and fundamental part of the development of many unique processes. Modern methods must be used in process engineering for tasks including cell quantization, pH control, temperature monitoring by sensors and actuators, and gas transfer. It is crucial to select the optimum temperature control device based on the particular requirements of each application by evaluating the heat load because bioreactors rely so heavily on temperature management. The type of bioreactor you utilize will depend on the cell type, its shear tolerance, and how effectively it can grow and generate. Engineering-wise, bioreactor technology has evolved greatly over time, allowing for more precise control of the microenvironment's numerous characteristics as well as real-time sensing and imaging capabilities that increase control and reproducibility of tissue graft formation. Deeper partnerships are also required between medical professionals, scientists, engineers, other stakeholders like regulatory bodies, and commercial partners to integrate the multiple requirements required for a clinically relevant bioreactor.

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