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Spinner Flask Bioreactor in Tissue Engineering

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Abstract:

Spinner Flask Bioreactors are usually made up of glass or plastic vessel which have been widely used in Tissue Engineering from production of articular cartilage to production of osteoblast cells that help in bone regeneration. Cartilage grown in spinner flask bioreactors had more cells and less GAG than the other types of bioreactors in tissue engineering. In recent years, these bioreactors have also been used for the invitro cultivation of human tenocytes and MSCs. In this type of bioreactor, the cell/scaffold constructs are connected to vertical needles striking from pinnacle of the vessel and immersed inside the culture medium. The pinnacle or the top part of this bioreactor is used for gas exchange and medium oxygenation. Mixing of the medium is maintained with a stir bar at the lowest of the vessel or different blending mechanisms. Spinner Flask Bioreactors have grabbed increased attention in recent years due to its wide range of Tissue Engineering applications. In this review, we have tried to explore the different domains where these bioreactors have found enhanced applications.

Keywords: Spin Flask Bioreactor Tissue Engineering**,** Cell Seeding**,** Bone Tissue Engineering, Articular Cartilage

1. INTRODUCTION:

Tissue Engineering (TE) is a study to develop techniques for in vitro production of tissue structures exhibiting tissue-specific morphological, biological, chemical, mechanical properties, and functions similar to in vivo [1].

Under in vivo conditions, tissues are implanted in complex micro and macro environments and exchange information with the entire organism, which determines tissue-specific functions [2,3]. In vitro tissue-forming constructs must mimic these in vivo conditions. Similar to the food industry, TE uses an advanced bioreactor system to control process parameters to ensure optimal culture conditions. Tissue generation begins with the separation of cells from a biopsy, the proliferation of cells in vitro, the generation and maturation of 3D constructs, and finally the use of the constructs as a system or graft in several

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individual process steps. It will be configured. Robustness, reliability and efficiency of a TE cycle is improved with the help of bioreactors.

During cell expansion, bioreactors help in reducing costs and improving process efficiency by fulfilling labor intensive manual work. While providing a 3D culture environment, mass transport mechanisms and cellular cross talk is more in conjunction to that in vivo. The transport of nutrients is also affected. Sufficient nutrient supply to a complex 3D structure is a huge challenge in TE and is the limiting factor for the size of any bioartificial tissue and embedding of a construct [4]. Cell viability is maintained by a dense vascular network with a mesh size of approximately 100-200 µm [5]. Therefore, tissue formation with high metabolic activity is not only based on diffusion-based passive mass transfer [6], but for active processes such as perfusion and convection [7]. Therefore, switching from 2D to 3D requires switching from static to dynamic culture conditions for most tissues. After seeding a proliferated cell population into a tissue-specific scaffold, the tissue constructs produced usually require a tissue maturation phase. This is the period during which cells adapt to the 3D environment, generate ECMs, and establish cell-cell interactions. Aside from cell-cell, extracellular matrix interactions, and soluble factors, physical stimuli such as mechanical, electrical, and electromagnetic cues are essential for establishing tissue function. For example, does vascular remodeling require shear stress [8-10], or mechanical stress determines the adaptation of the skeletal structure [11]. Electric fields have been established to be important triggers for healing wounds on the skin [12-14]. The main motivations for bioreactors are to provide an adequate supply of nutrients and oxygen, and to remove waste products and mimic conditions in the body [15-21] In addition to the scientific aspects, bioreactor systems can be a factor in the successful commercialization of TE processes. Further requirements such as ease of handling and compliance with good manufacturing practice can be addressed by the bioreactor system [22]. With reference to the TE paradigm, we will focus on the culture of cell types related to treatment and generally introduce bioreactors as tools for efficient cell growth. A step further in the TE cycle, bioreactors must meet basic requirements during tissue formation and maturation. We will also investigate the bioreactor system in the area of the musculoskeletal system.

2. CELL SEEDING ON 3D SCAFFOLDS

Cell seeding of scaffolds or the dissemination of remoted cells inside a scaffold, is the primary and main step in organizing a 3-D culture and can play a vital function in figuring out development of tissue formation [23]. Seeding cells into scaffolds at excessive densities has been related to stronger tissue formation in three-D constructs, which includes better prices of cartilage matrix production [24], multiplied bone mineralization [25], and stronger cardiac tissue structure [26].

Hence, engineering autologous grafts for medical packages the use of high initial cell densities, at the same time as restricting the biopsy length and/or the quantity of cellular expansion, calls for the cells to be seeded with the maximum feasible efficiency. Also, the preliminary distribution of cells in the scaffold after seeding has been associated with distribution of cells inside scaffold after seeding has been associated with the distribution of tissue eventually fashioned inside engineered constructs [25, 27-29], indicating that uniform cellular seeding may want to set up the idea for uniform tissue generation.

Significantly better efficiencies and uniformities had been attained while poly (glycolic acid) and non-woven meshes had been seeded in stirred-flask bioreactors [30]. Mixing the dilute cellular suspension round desk bound scaffolds suspended from mouth of the flask transports the cells into scaffolds with the aid of using convection.

Exploiting the precept of convective shipping for scaffold seeding, the glide of a cellular suspension at once via the pores of 3-D scaffolds the use of a multi-pass filtration seeding method produced extra uniformly seeded scaffolds as compared with static seeding [31]. When direct perfusion became included into an automatic bioreactor for three-D-scaffold seeding, better seeding efficiencies and extra uniform cellular distributions had been acquired in contrast to with both static seeding or the stirred-flask bioreactor [32]. Varieties of scaffolds may be efficiently seeded in an automatic and managed procedure the use of this idea and a simple bioreactor. Moreover, perfusion seeding may be effortlessly included right into a perfusion bioreactor device able to sport out each seeding of the scaffold and next culturing of construct. These seeding and culturing bioreactors were designed for engineering vascular grafts [33] and feature currently been utilized in engineering cartilage [34] and cardiac [35] tissues, and in keeping hepatocyte characteristic inside three-D scaffolds [36]. These structures now no longer most effective streamline the engineering procedure however additionally lessen protection dangers related to the managing and shifting of constructs among separate bioreactors.

3. SPINNER FLASK BIOREACTORS

Spinner flasks are easy bioreactors fabricated from glass or plastic vessel wherein cell/scaffold constructs are connected to vertical needles striking from pinnacle of the vessel and immersed inside the culture medium (Fig.1). The pinnacle or the top part of this bioreactor is used for gas exchange and medium oxygenation. Mixing of the medium is maintained with a stir bar at the lowest of the vessel or different blending mechanisms. The convective forces generated whilst stirring annihilate the nutrient attention gradients on the floor of the cell/scaffold constructs and convey turbulences which beautify mass delivery toward the center of the samples [37]. The complete machine is positioned in an incubator controlling temperature and oxygen content. The diploma of shear pressure is based on stirring speed. Spinner flasks had been first of all used to aid big biomass growth [38] and feature currently been exploited for tissue engineering applications, which includes the lifestyle and maturation of bone tissue substitutes the use of human osteocompetent cells derived from grownup tissues.

A look at via way of means of Karageorgiou et al. investigated the consequences of dynamic situations on constructs of human trabecular bone cells and BMP2-loaded silk discs (4mm in diameter and a pair of mm in thickness) in vitro after 28 days and in mouse calvaria defects after a 5 week implantation period [39]. Although the dynamic situations appeared to sell formation of bone-like tissue in vitro, no giant variations in bone formation capacity in vivo had been located among constructs cultured under dynamic and static situations. Later on, Kim et al. concluded the useful consequences of dynamic lifestyle in spinner flasks(50rpm) on BM-derived hMSCs seeded onto big aqueous-derived macroporous silk-scaffolds (15mm diameter and 5mm thickness) for eighty-four days [40]. In assessment to static controls, constructs cultured under dynamic situations confirmed higher cellular proliferation, osteogenic differentiation and mechanical properties, as glaring from improved expression of bone-precise genes. ALP activity, deposition of mineralized matrix and formation of prepared bone-like structures.

Spinner flasks showcase better capacity to enhance mass delivery in comparison to rotating wall vessels and may be used to impart better values of pressure to the cells. Spinner flasks may be used to engineer skinny bone substitutes for the reconstruction of flat bones or as bone patches for restorative applications. [41]

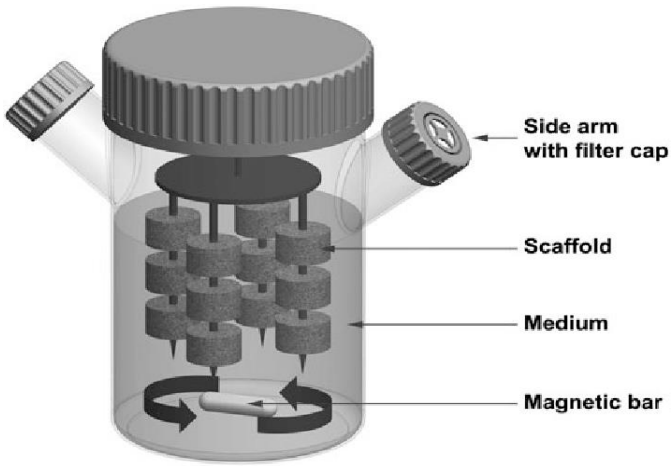

Fig.1 Schematic Representation of a Spinner Flask Bioreactor [41]

4. FLUID MECHANICS OF SPINNER FLASK BIOREACTOR:

Spinner Flask Bioreactors have been employed to produce articular cartilage in vitro as well as bone tissue engineering. The resulting tissue that is grown within the bioreactor depends on a wide range of parameters. The mixing rate, mass transfer, stress level and flow regimes are some of the most important factors on which can affect the morphology and biochemical composition of the resulting tissue and are determined by the hydrodynamics of the bioreactor [42]. It has also been reported that experiments involving bovine chondrocytes seeded and grown for 8 weeks under dynamic conditions in a turbulent spinner flask bioreactor, were thicker, more heterogeneous, stiffer, and contained more cells than those grown under static conditions [43]. Moreover, the hydrodynamic environments existing within the spinner flask bioreactor were compared with that of a rotating wall bioreactor in the case of articular cartilage invitro. Cartilage grown in spinner flask bioreactor had more cells and less GAG than those grown within the rotating wall type [44].

Freed and Vunjak-Novakovic et al calculated the Reynolds number for the bulk flow within a spinner flask bioreactor using the formula:

$$Re_p = \frac{N_p L_p^2}{v_p} \qquad \ldots.(1)$$

The Reynolds number was calculated to be 1758 which is greater than 1000. This signified turbulent flow. By comparing the flow conditions of prototype bioreactor with a model bioreactor, the following formulae have been designed for the determination of fluctuating velocity u(x,t), mean velocity $\bar{U}$(x,t) and mean shear stress $\bar{\tau}_{ij}$ of the bulk flow within a spinner flask bioreactor have been listed below [42]:

$$u(x,t) = U(x,t) - \bar{U}\,(x,t) \qquad \ldots(2)$$

$$\overline{U_p}\,(x,t) = \left(\frac{v_p L_m}{v_m L_p}\right) \overline{U_m}\,(x,t) \qquad \ldots(3)$$

$$\overline{\tau_{ijp}}(x,t) = \frac{(\mu_p v_p L_m d_m)}{\mu_m v_p L_m d_p} \overline{\tau_{ijm}}(x,t) \quad \ldots(4)$$

Where the suffix p denotes the parameters in the prototype bioreactor and m denotes those in the model bioreactor, μ, v, L and d represent the viscosity of the medium, velocity of the fluid stream, length of the bioreactor and diameter of the bioreactor respectively.

5. SPINNER FLASK BIOREACTORS IN BONE TISSUE ENGINEERING:

Reconstructive surgery is often the method of choice in case of large bone defects resulting from trauma, tumor infections or congenital abnormalities [45]. At present, the effective treatments for bone defects include autograft transplantation, allograft transplantation or the use of synthetic materials like metals and bone cements [46]. But the severe limitations of these processes have paved the way for development of new bone tissue engineering strategies [47]. In this process, biodegradable porous scaffolds made from natural or synthetic biopolymers [48-50] are seeded with pluripotent marrow stromal cells (MSCs) from the patient's bone marrow [51]. These MSCs can give rise to osteoprogenitor cells which can further enhance new bone formation [52]. With the addition of proper growth factors and maintenance of proper cultured conditions, these cells can then be transplanted to the defective site [53-57].

For the cultivation of synthetic tissues for in vivo implantation, several bioreactor designs have been reported. Some of these bioreactors produce convective forces, which can help to overcome nutrient transport constraints. Scaffolds are fastened to needles suspended from the flask's lid. A magnetic stirrer bar generates convective forces that allow the media surrounding the scaffolds to be continuously mixed. Seeding cells on scaffolds [58], culture of cell/polymer constructions for cartilage [59-60], and bone tissue regeneration have all been done in spinner flasks [61]. In the laboratory, bone tissue engineering was done by preparation of porous PLGA scaffold, isolation of rat MSCs, cell seeding in the scaffold and culture of cell/polymer scaffold construct in a spinner flask bioreactor (Fig.2).

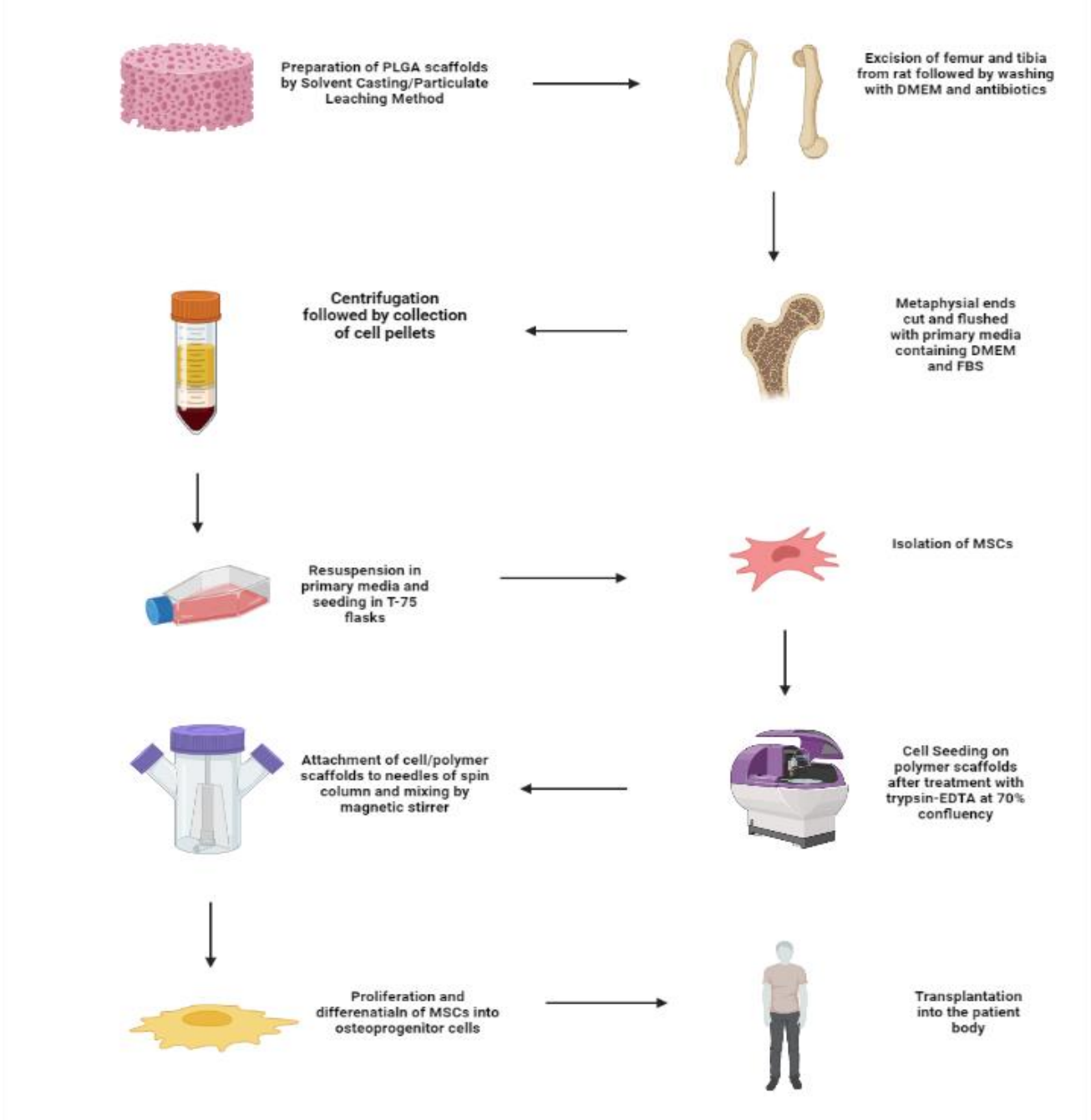

Fig.2 Procedure of using Spinner Flask Bioreactor in Bone Tissue Engineering

6. CULTIVATION OF HUMAN TENOCYTES USING SPINNER FLASK BIOREACTOR

Tendon ruptures remain a major challenge in orthopedics [62-63]. Healing of tendon injuries takes time and causes scarring as a functionally altered repair tissue [64]. Tendons are low blood vessels and low cell tissues with a low metabolic rate [65]. Tendon cells, which are resident cells, are embedded in abundant

ECM [66]. This ECM is mainly composed of type I collagen arranged in tight parallel bundles of fibril [67]. The expression of type III collagen increases with tendon healing [68]. The main proteoglycan in the tendon is decorin [69-70]. However, aggrecan and glycoprotein COMP are also present in small amounts in the tendon ECM [70-71].

In recent years, bioreactor culture methods using microcarriers have been intensively studied for chondrocyte proliferation [72-73]. They provide a suitable basis for the continuous growth of cells without the passage of trypsin / ethylenediaminetetraacetic acid (EDTA) or other agents required for cell ablation. It leads to "metabolism" and the loss of newly synthesized ECM. In addition, human chondrocytes can be cultured on microcarriers while maintaining the chondrocyte phenotype [74]. In addition, bioreactor cultures are less time-consuming, costly, and labor-intensive than monolayer cultures because cell proliferation can be achieved by simply adding empty microcarriers [75]. No loss of newly synthesized cell-related ECM occurs in microcarrier cultures. In addition, bioreactor culture has the advantage of enabling regulation and automation using an advanced bioreactor system to monitor the physical, chemical, and biological environment. The agitation required for permanent flotation of the microcarriers ensures mechanical stimulation of the culture.

Tendon healing is a time-consuming process that results in the formation of functionally altered repair tissue. Reconstruction of tendons based on tissue engineering is of increasing interest. Tendon cells from human hamstring tendons were grown in monolayer cultures prior to seeding into Cytodex ™ type 3 microcarriers at two different seeding densities (2.00 and 4.00 3106 cells / 1000 cm2 surface). Tendon cell vitality, growth kinetics, and glucose/lactate metabolism were determined as a function of inoculation density and agitation rate (20 or 40 rpm) in a spinner bottle bioreactor over a 2-week period. Gene expression profiles of extracellular matrix (ECM) tendon markers (type I / III collagen, decorin, oligomeric cartilage protein [COMP], aglycans) and tendon marker tendon markers were analyzed using real-time detection polymerase chain reaction (RTDPCR). did. Deposits of type I collagen and decorin are detected by immune labels. Tenosite adhered to the carrier, maintained vitality, proliferated, and showed increased glucose consumption and lactation formation under all culture conditions. The "bead-to-bead" migration of cells from one microcarrier to another is a prerequisite for continuous tendon cell enlargement and has been demonstrated by scanning electron microscopy. Gene expression of type I and type III collagen was largely unaffected, but an expression of aggrecan and partially decorin and COMP was significantly downregulated compared to monolayer cultures. The expression of sclerosing genes did not show significant regulation to carriers. In summary, tendon cells were able to proliferate well on microcarriers. Therefore, the bioreactor is a promising tool for the continuous expansion of tendon cells [76].

7. FABRICATION OF ENGINEERED CARTILAGE

Osteo chondral defects caused by degenerative joints and wounds cause great pain to many patients and are becoming more and more problematic. The ability of cartilage to repair itself is very limited due to its simple structure and lack of neurovascular and lymphoid tissue. Although existing clinical practices for repairing osteochondral defects and wounds are diverse, they are not ideally effective in completely repairing cartilage tissue [77-79]. Given the rapid development of cartilage tissue engineering, this approach appears to be very promising in the repair of osteochondral defects in clinical practice. Since the production of constructs with a combination of chondrocytes and the appropriate scaffold is important for cartilage tissue engineering, the choice of the appropriate cell source and supporting scaffold determines the success rate of the artificial tissue [80-81]. Recently, adipose tissue-derived stem cells (ADSCs) are more pluripotent, in vitro proliferative, accessible, pain-relieving, and for minimal reasons than interstitial mesenchymal stem cells (BMSCs). It has become a much more attractive source of cells for tissue engineering. Immunological rejection [79]. And now it is used by many researchers to produce tissue-engineered cartilage [82-83].

The selection and design of cartilage scaffolds as the backbone of cartilage by tissue engineering is important for the successful repair of damaged cartilage tissue. Many scaffolds are involved in current research in our field, but they all have advantages in their applications and at the same time have some weaknesses that become apparent [84]. Improved methods for reducing the weaknesses of existing frameworks consist primarily of material recombination or surface modification. Chitosan [85], which is similar in structure to the cartilage substrate glycosaminoglycan (GAG) and has a degradation product that is a glucosamine monomer, has no adverse reactions to the human body and has many attractive properties including excellent biocompatibility. It is a high molecular weight natural polymer with characteristics. Degradable, non-toxic, immunogenic, anti-inflammatory, antibiotics, etc. The degradation product of collagen, gelatin, has strong biological activity and promotes chondrocyte adhesion, proliferation, differentiation, and extracellular matrix secretion on the scaffold [86]. Currently, these two natural biopolymers have been combined as cartilage scaffolds by several researchers [87-88]. However, their physical properties and cell proliferation profiles on the scaffold can differ significantly due to different manufacturing conditions. Here, the advantages of the hybrid framework are preserved, but the disadvantages of each are overcome. This is achieved by adjusting the ratio of the two components to adjust and control the performance of the scaffold. Finally, the formation of the optimal content of chitosan/gelatin hydrogel is determined using the lyophilization method as a scaffold for the construction of artificial cartilage.

When constructing tissue-engineered cartilage, an in vitro culture environment is also an important consideration to ensure the success of the artificial tissue given the appropriate seeded cells and scaffolding material. With growing interest in cell culture techniques and detailed research, researchers are beginning to recognize the advantages of the dynamic environment of bioreactors over traditional static cultures for tissue engineering in cartilage production [89-91]. Surprisingly, the simple design of the spinner flask can provide a dynamic microenvironment by the flow of liquid generated by agitation to promote mass transfer and promote cell proliferation and differentiation. increase. Its simplicity and functionality have been proven in a wide range of applications in the field of tissue engineering [92-93]. In 2001, Gooch, etc. seeded cartilage cells in a PLLA scaffold [94] and cultivated them in spinner flasks. As a result, collagen and glycosaminoglycan secretion is increased by mixing during agitation. In 2010, Zhu, etc. [95] showed that spinner flask cultures improved the survival, proliferation, and expression of certain genes in ADSC.

8. HEMOSTASIS OF HUMAN MSCs

Over the last quarter-century, interest in human mesenchymal stem cell (hMSC) research has accelerated due to its potential in regenerative medicine, tissue engineering, and cell-based therapies. hMSC exhibits a wide range of attractive properties, including B. Pluripotent differentiation [96], secretion of cytokines that support cell survival, regulation of immune response [97], and hypoimmunogenicity [98]. Promising preclinical results have led to a surge in clinical trials over the last decade, with more than 1100 trials using hMSC for the treatment of graft-versus-host disease, diabetes, myocardial infarction, and other diseases. It has been. Multiple tissue sources such as bone marrow, diabetic tissue, pulp, and umbilical cord can be used to isolate hMSC. Although most studies focus primarily on bone marrow-derived hMSCs, adipose tissue-derived hMSCs appear to be a promising alternative and have high isolation yields [99-102]. Regardless of tissue source, hMSC yields are orders of magnitude lower than the minimum effective intravenous dose of 70,190 million cells used in clinical trials [103]. Therefore, large-scale in vitro growth is required to provide sufficient cell numbers. In addition, as stem cell products become viable off-the-shelf products beyond the experimental stage, in vitro expansion systems need to be scaled up under current Good Manufacturing Practices.

A system that demonstrates the potential for 1: 1 scale production of hMSC while preserving stem cell properties is a microcarrier-based suspension bioreactor [104-106]. Lawsonnet. Al. Recently, a 50 L agitation tank bioreactor retains the ability to regulate trillin differentiation, surface marker expression, and immune response, as evidenced by its ability to suppress T cell proliferation via indoleamine-2,3. Showed a 43-fold enhancement of hMSC-dioxygenase (IDO) induction [104-106]. Suspension bioreactors have a high surface-area-to-volume ratio, which allows them to achieve a high degree of expansion. Since hMSC is an attached cell type, proliferation is directly affected by the available surface area. In suspension bioreactors, microcarriers are suspended in the medium by stirring, providing the surface area available for hMSC expansion [99,107]. Various microcarriers with diameters of 100-300 μm, synthesized from materials such as polystyrene, dextran, polyvinyl alcohol, and glass, are commercially available, depending on the intended use [108-110]. Additional protein coatings can be applied to microcarriers to promote cell adhesion. B. Collagen, cellulose, fibronectin, etc. Microcarriers also can be fabricated using porous materials to increase surface area for cell growth, however, this may reduce the efficiency of downstream hMSC harvesting.

The microenvironment of a floating bioreactor differs from the static planar culture in the dynamic forces involved. Constant movement through the impeller causes fluid flow and shear stress. Different agitation rates have been used in many studies, depending on the type of microcarrier and the shape of the vessel and impeller [107,111,112]. However, it is common practice to use the lowest agitation rate required to suspend the microcarriers in the medium so as not to alter gene expression or damage cells. Ismadiet. al. Implemented a particle image velocimetry method to quantify the velocities and shear stresses that occur at various locations within the spinner piston bioreactor [113]. The authors found that fluid velocities and shear stress varied throughout the reactor, with high speeds measured near the side walls of the flask and near the bottom of the flask at the tip of the impeller. Similarly, the location of the maximum shear stress was near the bottom of the flask. Increasing the agitation rate from 10 rpm to 80 rpm linearly increased the average velocity, average shear stress, maximum velocity, and maximum shear stress.

As the hMSC phenotype is sensitive to changes in the microenvironment, previous studies investigating and optimizing the therapeutic efficacy of hMSCs in stationary planar cultures may differ from those of a conventional bioreactor. suspensions, as some characteristics of hMSCs, are known to be altered under shear force. For example, shear stress has been shown to enhance bone differentiation [114]. Similarly, upregulation of chondrogenic genes in cultures exposed to shear stress was observed along with an increase in extracellular matrix (ECM) deposition and type II collagen secretion [115]. In addition to differentiation, shear stress also affects the immunoregulatory capacity of hMSCs [117]. Diaz and. al. showed that the introduction of hMSCs into the fluid increased the secretion of prostaglandin E2 (PGE2) as well as the ability to inhibit the production of tumor necrosis factor (TNF) α. This distinction between stationary planar cultures and bioreactor cultures in spinner flasks, although not fully understood, has been recognized [99]. Although agitation can fundamentally alter basal metabolic processes, very few studies on sliding stress on cellular senescence have been reported [117].

9. CONCLUSION:

Tissue Engineering (TE) aims to repair, replace, or regenerate tissues or organs by transforming basic knowledge of physics, chemistry, and biology into practical and effective materials or devices and clinical strategies. It is a rapidly evolving field [118]. Cell seeding is the spreading of cells in a culture vessel for cell culture activity. For attached cells, this means using the appropriate material or, in some cases, adding the cell suspension to a surface-treated culture vessel [119]. Cell dissemination is the first step in cell adhesion, and its efficiency and distribution can affect the ultimate biological performance of the scaffold. One of the factors that contributes to maximizing cell seeding efficiency and consequent cell adhesion is scaffold design [120]. For cell seeding of 3D scaffolds under dynamic conditions, spinner flasks are one of

the simplest and most widely used bioreactors. Spinner flasks are used as bioreactors to grow suspension cultures in liquid media. These flasks are agitated tank bioreactors, where impeller mixing keeps cells floating and fluid movement helps mass transport of nutrients and waste. The Spinner flask bioreactor was used to generate articular cartilage in vitro. The dynamic environment of bioreactors is known to have a profound effect on tissue growth and development [121]. Tissue engineering is emerging as a potential alternative to current treatments for bone damage and defects. However, the usual tissue engineering approach has some obstacles to the development of functional tissues, including inadequate nutrient and metabolite transport and uneven cell distribution. Culturing bone cells in a three-dimensional structure in a bioreactor system is a solution to these problems as it improves mass transfer in the culture system. Spinner flasks, rotating wall vessels, and perfusion systems were examined for bone tissue engineering, based on which variations supporting cell dissemination and mechanical stimulation were also studied [122]. Tendon healing is a time-consuming process that results in the formation of functionally altered repair tissue. Reconstruction of tendons based on tissue engineering is of increasing interest. Expression of sclerosing genes did not show significant regulation to carriers. In summary, tendon cells were able to proliferate well on microcarriers. Therefore, the bioreactor is a promising tool for the continuous proliferation of tendon cells [123]. Biological treatments using artificial osteochondral composites are gaining increasing attention for the repair of cartilage defects. Osteochondral composites combined with dynamic culture are great for improving the quality of constructs and cartilage regeneration as dynamic conditions that mimic the in vivo conditions in which cells are constantly exposed to mechanical and chemical stimuli offers the possibility [124]. Dynamic culture revealed preferred extracellular matrix production, especially in hydrogel scaffolds. In addition, enhanced mass transfer contributed to interface formation, cell infiltration, and distribution in osteochondral composites [125].

10. DECLARATION

The authors declare that they have equal contributions to the completion of this paper. They have no competing interests. This work has received no specific grant from any funding agency.

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