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# Review on bioreactors in tissue engineering

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## ABSTRACT

Tissue engineering is an exciting field that aims to create regenerative alternatives to harvest tissues for transplantation. In this move towards success needs delivering the tissue progenitor cells over biocompatible three dimensional (3D) scaffolds (natural or synthetic). It is clear that scaffold design is increasing in complexity and becoming smarter. The only obstacles to the generation of functional tissues and their widespread clinical use are related to a limited understanding of the regulatory role of specific physico-chemical culture parameters on tissue development and the high manufacturing costs of the few commercially available engineered tissue products. By enabling reproducible and controlled changes of specific environmental factors, bioreactor systems provide both the technological means to reveal fundamental mechanisms of cell function in a 3D environment and the potential to improve the quality of engineered tissues. In this context we evaluated various sophisticated dynamic bioreactors for fabricating organs of given clinical application. In comparison with other bioreactors perfusion system is amenable to multiple tissue engineered construct production, uniform tissue development, and yet is simple to operate and can be scaled up for potential clinical use.



## KEYWORDS

Tissue engineering;
Polymer scaffolds;
Bioreactors;
Static cultures;
Dynamic cultures.

## INTRODUCTION

Tissue engineering (TE) represents one of the major promising fields in modern medicine. Tissue engineering combines different disciplines ranging from biology and material sciences to engineering and clinical disciplines. The aim of tissue engineering is the development of therapeutic approaches to substitute diseased organs or tissues or improve their function[1]. In Tissue Engineering three major strategies are used to control the regeneration of damaged tissues. First is the implantation of an acellular matrix to stimulate formation of new tissue[2]. In vivo studies have shown that it is difficult to induce cell migration into the scaffold, often resulting in poor tissue formation. The second is promotion of the self-assembly of cells, although much effort and several studies have been carried out, no functional tissue has yet been regenerated with this method. A lack of cohesion between cells, dedifferentiation, and an inadequate resulting tissue shape are among the main limits[3]. Therefore, the last strategy consists of using a scaffold that offers the possibility of tailoring the initial properties of

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the construct and allows an easier application of mechanical constraints on the young and fragile construct at the beginning of the regeneration. This approach implies seeding of appropriate cells on to required scaffold material (natural or manmade, biodegradable or not) shaped to obtain an appropriate geometry.

During the 1990s, TE progressed rapidly and biological substitutes were developed for several tissues and the products reached the market little over a decade. The present status of research and development (R&D) in tissue engineering industry costs over $3.5 billion worldwide[4]. However, tissue engineering industry is not successful in meeting the needs of millions of people waiting for transplants and in last five year approximately ten thousand people died while on the waiting list[5].

The growing demand shifts the emphasis from organ shortage to scale up of tissue transplants and synthesis of bio-inert materials to regenerate the damaged tissues. Issues of scale-up present additional challenges in tissue engineering strategies i.e., 3D scaffold which is successful in small scale may fail in larger scale applications where nutrient diffusion into the center of an extensive cell based construct may be limited during the initial stages of healing before any appreciable angiogenesis[6].

Even the most successful tissue engineering products will need to demonstrate cost-effectiveness & cost-benefits over existing therapies, must assure complete safety to the patients and fulfillment of growing rigid framework in terms of quality control and good manufacturing practice. In these situations bioreactor-based tissue constructs are more attractive[7]. A tissue engineering bioreactor can be defined as a tool that uses

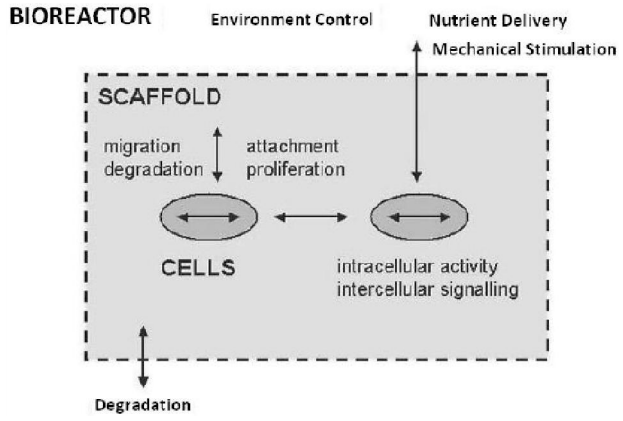


**Figure 1 : Schematic of tissue engineering processes**

mechanical means to control biological processes. Bioreactors in tissue engineering are very efficient in seeding the cells on 3D scaffolds, improved mass transfer in 3D cultures and automation of media and gas exchange in cell microenvironment[8]. Bioreactor systems also offer the possibility to investigate cell function, cell interactions and tissue development within controlled 3D models, which may be designed to recapitulate specific aspects of the actual *in vivo* environment[9].

In tissue engineering, application of bioreactors may be found in several areas. At the outset, we need bioreactors for expansion of cells for direct transplantation at the damaged site, for example haematopoietic stem cells and mesenchymal stem cells and mature red blood cells, etc[10]. Secondly, we use bioreactors to grow 3D tissues prior to implantation, eg skin, cartilage, bone, blood vessels etc.

## Bioreactor design requirements

There are certain principles which have to be adhered when developing a tissue engineering reactor. The material selection for reactor design is very crucial which ensure the materials do not bring any adverse reactions in the cultured tissues. Any material which is in direct contact with media or tissues must be biocompatible or bioinert[11]. Metallic alloys like stainless steel best suits for reactor design due to its resistance against corrosion and leaching. Various low cost plastics are also found to be very useful in rector design, but there are certain limitations in this material which have to keep in mind. The reactor parts must be sterilised if they are to be re-used and it is done by autoclaving or disinfected by sterilants like alcohol. If they have to be autoclaved, materials that can withstand high temperature and pressure must be used in bioreactor design. We often see transparent materials like glass in bioreactor design, which can be easily sterilsed by autoclaving or by alcohol swabbing. However, materials with diverse properties are needed for various components in the bioreactor. For example, transparent material allows the construct to be monitored in the bioreactor during culture while elastic tubing can help with assembly of the bioreactor.

In recent times, need to culture particular tissue types have seen the introduction of more sophisticated reactor systems for replicating organs using biological scaffolds which support the cell proliferation. For a given

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clinical application culture conditions may need to be optimized with respect to cell source, scaffold material, media composition and fluid dynamics of the reactor system[12]. Thus we must realize the application of a single bioreactor for all cell and tissue culture operations is questionable. In this review our intension is to discuss various tailor made culture systems which are successfully in providing environment for specific tissue growth.

## BIOREACTORS IN TISSUE ENGINEERING

As an important component of tissue engineering, bioreactor system plays a significant role in providing an optimized environment for functional 3D tissue development. In this regard, variety of culture systems have been developed for supporting engineered tissue constructs. These culture systems can be fundamentally divided in to two main categories.

I. Static culture systems
II. Dynamic culture systems

### Static culture systems

In tissue engineering the most common and simplest culturing strategy is to cultivate a cell-seeded construct in static media. This static culture method is very conventional way of culturing cells by using sterile polypropylene petri-dish or well plates. In static cultures approximately 2X106 cells were seeded over tissue engineering scaffolds with the seeding efficiency of 50 to 55% and grown in a humidified CO2 incubator under static conditions. After seeding, scaffolds were allowed to plunge in culture medium for uniform supply of nutrients and media has to change every third day until we get consistent cell growth over the scaffold surface.

This traditional approach does not fulfill all the requirements for regeneration of every functional organ. When 3D scaffolds were grown in static culture, cells on the outer surface of the construct are viable and proliferate readily while cells within the scaffold may be less active or necrotic[13]. Continuous oxygen levels in the static 3D culture revealed that an oxygen gradient had formed from the surface of the 3D scaffold towards the center. After 5 days the oxygen concentration dropped to 0 % in the center of the scaffold and 4 % in the surrounding medium[14]. In the obscene of vascular blood supply *in vitro*, gaseous exchange and nutrient delivery to the cells throughout 3D tissue engineering scaffold occur by passive diffusion (up to 100 μm only). As a result thin tissues like skin (100μm thickness) may be readily grown *in vitro* than thicker vascular tissues such as bone. In dynamic culture conditions, the stimulus from mechanical forces like hydrostatic pressure and shear generated by the media flow can be more beneficial which drives cell constructs to a more *in vivo* like conditions. The figure given below reveal higher numbers of dead cells under static conditions compared with the dynamically cultured scaffold.

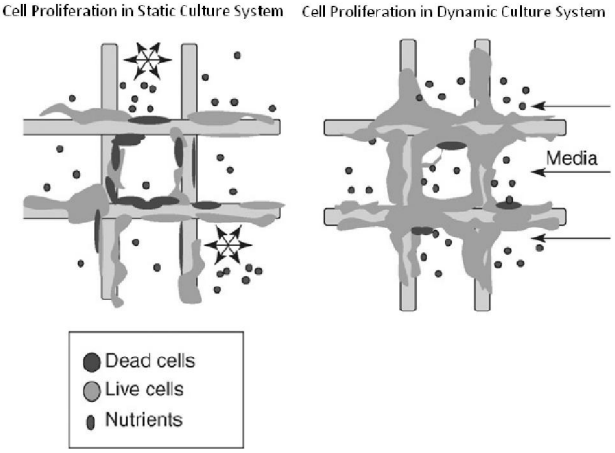


**Figure 2 : Cell growth under static and dynamic culture systems**

### Dynamic culture systems

Despite burgeoning advancement in tissue engineering, mass transfer limitation remains a prevalent predicament[15]. Dynamic culture systems using various engineered bioreactors can be an alternative for enhancing mass transfer and reinstating the *in vivo* physiological fluidics *in vitro*[16]. Tissue culture reactors those engage dynamic media flow for developing 3D tissues as fallows;

(A) Rotating-wall vessel bioreactors
(B) Spinner flask bioreactor
(C) Concentric cylinder bioreactor
(D) Flow perfusion bioreactor

### (A) Rotating-wall vessel bioreactors

Rotating wall bioreactor has been first developed by NASA/JSC for growing anchorage dependent cells in microgravity environment. When this bioreactor was first tested on Earth, cells started aggregating and form structures resembling tissues. These observations led to the possibility that the bioreactor might be used to study co-cultures of various cell types and the association of pro-

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liferation and differentiation during the early steps of tissue development[17]. Rotating wall vessel bioreactor is characterized by a permanent rotating culture chamber with a gas exchange membrane, the rotation speed of which is adjustable to produce a free-falling state. During rotation the centrifugal force, fluid shear and gravitational force acting on the scaffold sums to zero that make the scaffold suspended in the culture medium[18]. This seems to encourage the uniform growth of the tissues, thus promoting uniform cellular interactions. It also protects fragile tissues from cracking because it decreases mechanical stresses, including shear stress, and it limits the impact of cells on the walls of the bioreactor.

These rotating wall bioreactors are horizontally rotated with fluid filled culture vessels (zero headspace) and are oxygenated through a silicone rubber membrane by an air pump that draws incubator air through a 0.22 μm filter. The initial rotation speed of the reactor was adjusted so that culture media, individual cells and pre aggregated cells over the scaffold surface rotate synchronously with the vessel, thus providing efficient mass transfer and low media wastage[19]. On the other hand, as cells grow and form aggregates, rotation rate has to be increased to maintain cells in suspension and microgravity conditions.

Rotating wall vessel reactor competently suspends the polymer construct (typically PGA or PLLA-PGA) and creates Reynolds numbers more conducive to a minimal boundary layer which ultimately boosts mass transfer in the constructs[20]. Cartilage, heart muscle, skeletal muscle, pancreatic islets, liver and kidney are few of the normal tissues being grown in rotating wall bioreactor.

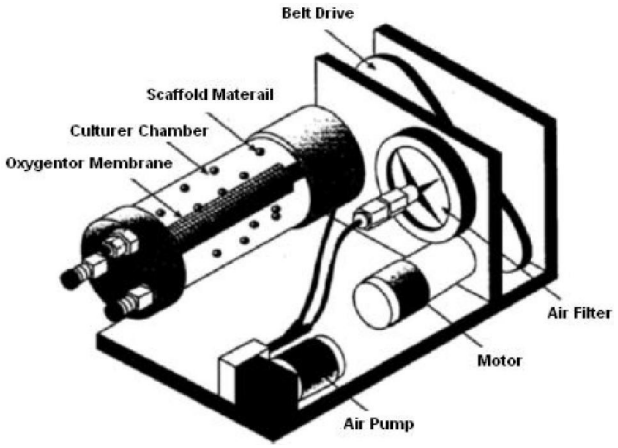


**Figure 3 : Rotating wall vessel bioreactor**

## (B) Spinner flask bioreactor

Chondroblast are the specialized cells to construct cartilaginous tissue which is a connective tissue found in many areas of human body. Chondroblasts that get caught in the cartilaginous matrix are called chondrocytes. These chondrocytes synthesize large amount of extra cellular matrix (ECM) and are very different from other living cells. Although autologous chondrocyte implantation has already been in clinical application, chondrocyte dedifferentiation is problematic during proliferation culture[21]. Only similarities they share with other cells are their basic needs: the delivery of nutrients and the removal of metabolites. Therefore, the culture conditions should enable adequate transfer of nutrients and oxygen along with removal of wastes. Spinner-flask bioreactor is one such device that has been designed to better control the redifferentiation of de-differentiated cartilage tissues *in vitro*.

Spinner-flask bioreactor is the most common mechanically stirred bioreactors that can be used for the seeding of cells on 3D polymer scaffolds and for subsequent culture of the constructs. A spinner flask bioreactor uses a magnetic stirrer to mix the cell suspension around a static scaffold, aiding in the cell allocation throughout the scaffold[22]. Polymer scaffolds such as biodegradable PGA are threaded onto needles, separated by spacers. These provide attachment sites for chondrocytes and promote cell migration and differentiation. In this bioreactor cells are aimed into the scaffold by convection. Constant stirring of culture medium overcome the diffusion limit and the scaffolds are exposed to fresh nutrients and oxygen all the times. Nutrient medium in spinner flask has to exchange every day, for which cell aggregates were allowed to settle and 50% of the culture supernatant was collected and centrifuged. After the exhausted medium was removed, the centrifuged cells and fresh medium were added to the spinner flasks.

Though spinner flask bioreactor technique is effective for cartilage tissue engineering, this strategy does not appear to provide internal nutrient gradients conducive to colonization of scaffold interiors in other tissue engineering areas[23].

## (C) Concentric cylinder bioreactor

It's a homogeneous bioreactor system also familiar as modified air-lift bioreactor. Concentric bioreactors are the object of much attention owing to their simple

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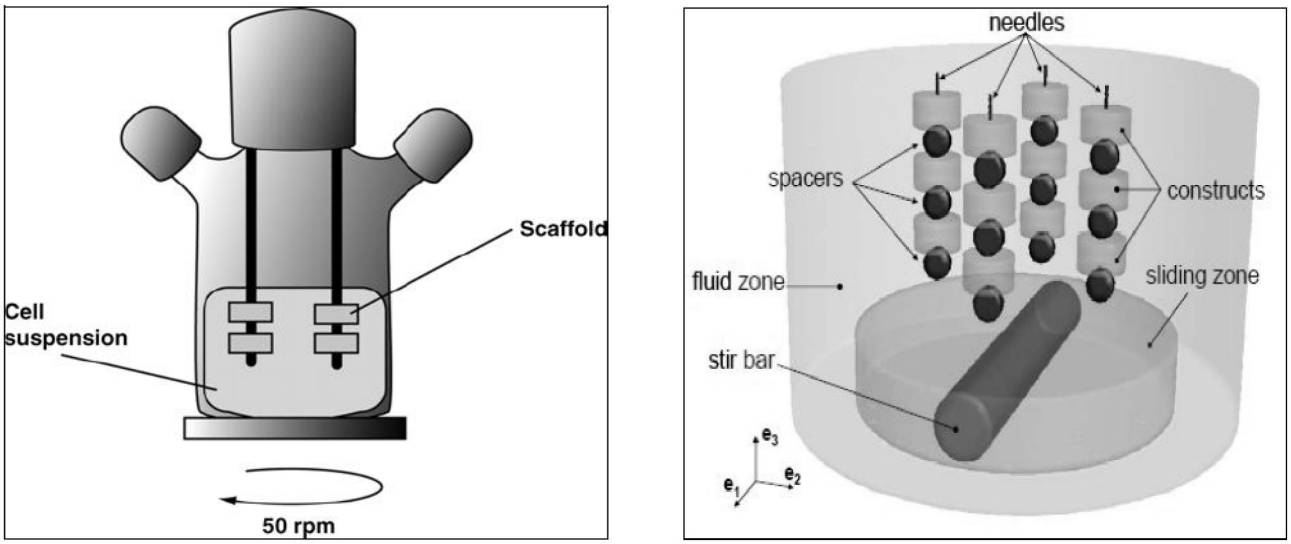


**Figure 4 : A and B spinner flask bioreactor**

construction and low energy consumption, together with high mass and heat transfer rates. These bioreactors are very useful for primary studies of construct growth and to assess the importance of cell density, nutrients, and hydrodynamic loading on cartilage development. Simple concentric bioreactor geometry will ensure low shear stress environment, uniform nutrient transport and dynamic seeding of scaffold[24]. The concentric cylinder bioreactor consists of a stationary inner cylinder and an outer rotating cup. The gap between the inner and outer cylinders is very narrow (2 to 3 mm). Porous scaffolds are positioned on the inner cylinder and protrude into the space between the inner and outer cylinder to facilitate cell seeding and nutrient transport[25]. Scaffold seeding efficiency for this reactor is also greater than 95%within 24 hours.

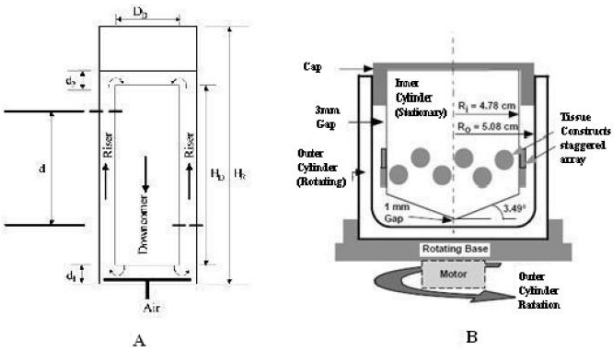


**Figure 5 : A and B schematics of concentric airlift reactor & diagrammatic explanation of concentric reactor in tissue engineering (Source: Timothy M.Wick & Tanya Farooque, 2009); whereas $D_D$- Downcomer diameter, $d_1$- Bottom clearance, $d_2$- Top clearance, $H_D$- Inner tube height, d- Pressure measurement distance, $H_R$- External tube height, $R_i$- Radius of internal cylinder, $R_O$- Radius of the outer cylinder.**

The concentric cylinder bioreactor is operated in a fed-batch mode. In this reactor scaffolds were spaced uniformly around the inner cylinder in rows for uniform nutrient supply[26]. Later on the bioreactor was assembled on the motor mount and placed in 5% $CO_2$ incubator. Reactor vessel rotational speed was adjusted such that constructs remain suspended close to a stationary point within the vessel, relative to an observer on the ground, due to a dynamic equilibrium between the acting gravitational, centrifugal, and drag forces. Medium is exchanged batch wise (at a rate of 50% every 2–3 days or 3 ml per construct per day) and is equilibrated with gas continuously[27].

## (D) Flow perfusion bioreactor

The last decade has seen several efforts at improving mass transfer limitations for 3D scaffolds. For example, cell-seeded porous scaffolds have been set up on orbital shakers, spinner flasks and rotating bioreactors, etc[28]. These methods increase media flow across the surface of the scaffold, offering an improvement over traditional static culture techniques. While these technologies satisfy the *external* requirement for medium flow, convection of medium at the external surface does not guarantee the media distribution within thick porous scaffold interiors[29].

In contrast to past expertise, we would like to present flow-perfusion bioreactor to meet the *internal* requirement for flow within the porous network of the scaffold. A flow perfusion culture offers several advantages, notably the ability to mitigate both external and

internal diffusion limitations as well as to apply mechanical stress to the cultured cells. Previous studies on tissue engineering scaffolds also proved that enhanced medium delivery, improved oxygenation and controlled shear may potentially increase cell differentiation[30]. The amount of shear stress experienced by cells cultured in a flow perfusion system can be varied simply by varying the flow rates through the system. Of course, depending on the porous structure, the local shear stresses experienced by individual cells will be variable and depend on the scaffold micro architecture[31].

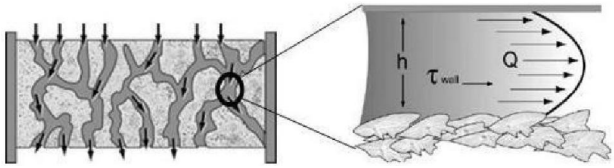


**Figure 6 : Flow perfusion culture and fluid shear on mono layer cell cultures in a flow chamber, where τ is shear stress, Q is flow rate, h is separation of parallel plates**

In a flow perfusion bioreactor, medium is pumped through each scaffold continuously, where medium is delivered through each cultured scaffold. Flow perfusion reactor with well controlled mechanical strains and dissolved oxygen tension provided an environment that better mimics the *in vivo* physiological features of the target tissue and supports cell scattering and growth as well as the differentiation of the cells into specialized lineages[32]. This particular reactor found very successful in seeding cells in to thick scaffolds whose critical depth is more than 2mm. Therefore, flow perfusion reactor found to be more appropriate for uniform media deliver to the core of bone tissue engineering constructs with higher thickness for consistent cell growth.

In this reactors medium is drawn from the first medium reservoir by the actions of the peristaltic roller pump (0.1 ml/min). The medium is then pumped downward through the flow chamber. On exiting the flow chamber it flows to the second medium reservoir[33]. Under the force of gravity, it then returns to the first medium reservoir, completing the cycle. The media in the reservoir was changed for every 2–3 days until process is accomplished. Diagram of the perfusion bioreactor system is provided as a supplementary figure.

The perfusion bioreactor system has superior performance over the static and other dynamic cultures and yet maintains the simplicity for operation and supports uniform functional implant development[34]. The implants

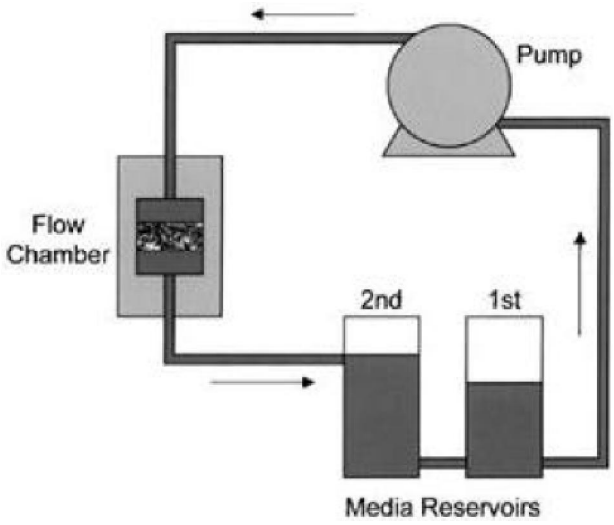


**Figure 7 : Flow perfusion system cyclic diagram**

grown in the perfusion system have uniform cell density and maintain their multi-lineage differentiation potential, which demonstrate that perfusion reactor system has imperative application in tissue engineering.

## DISCUSSION

Tissue engineering is a concept to fabricate autologous tissue constructs similar to native tissue for replacement and repair of injured tissue and even whole organs. One best approach to tissue engineering is to create an *in vitro* environment that provides the biochemical and mechanical signals to control tissue development and to create living constructs with a high degree of maturity before implantation. The ideal *in vitro* conditions for such tissue development are not exactly known, but it is confirmed that optimized cell distribution on scaffolds, a high level of sterility, an efficient cell culture medium and exposure to physical stimuli may be beneficial for developing tissue constructs. In this context we kept our focus on various bioreactors with their ideal conditions for fabrication of various 3D tissue constructs.

In this review we would like to assert that rotating wall bioreactor is best suited for growing fragile tissue due to its lower shear stress and provides more *in vivo* like conditions in the reactor. The fluid flow in rotating drum reactor can be tuned both to enhance nutrient transport to the growing tissue and to control the shear stresses experienced by the tissues. On the other hand spinner flask bioreactor can induce the fabrication of mechanically tough cartilage (chondrocyte aggregates) due to its recirculating flow patterns. Spinner flask re-

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**TABLE 1 : Advantages and limitations of various tissue engineering reactors**

| S.No | Bioreactor | Advantages | Limitations |
|---|---|---|---|
| 1 | Rotating Wall Bioreactor | a. Reduces the shear and turbulence generated by conventional stirred bioreactors.<br>b. Effective for culturing difficult primary cell lines which are fragile.<br>c. Culture cells in a more *in vivo* like environment. | a. The growth of heart and bone tissues found to be not uniform due to varying shear gradient across the rotating drum.<br>b. Rotating-wall reactors need control systems to vary the rotation speed of the vessel in function of the tissue size.<br>c. Change in gravity also makes the term" micro gravity" open to question. Since, rotating wall bioreactor was designed to perform best in space. |
| 2 | Spinner Flask Bioreactor | a. Much easier to clean and sterilize the whole reactor setup.<br>b. Through transparent reactor vessel, it is very much possible to monitor each and every step in the reactor closely.<br>c. Due its small size (100ml-5lit), media requirements and process cost can be minimized. | a. Recirculating flow patterns exerts centrifugal force that drives the suspended cells against the vessel wall, this hydrodynamic forces can damage the cells.<br>b. Addition of gases (oxygen) is inevitable for large scale spinner flask, which can damage the cells due to cell-bubble attachment. |
| 3 | Concentric Cylinder Bioreactor | a. The ability to seed scaffolds with cells under dynamic conditions.<br>b.Well-defined, uniform hydrodynamic loading of scaffolds.<br>c.Reactor handling is uncomplicated, no other manipulations except media exchange. | a. Culturing of metabolic very active and sensitive cell types such as hepatocytes is difficult. |
| 4 | Flow Perfusion Bioreactor | a. Perfusion bioreactor system is helpful for supporting long-term development of 3D engineered tissue constructs using porous scaffolds.<br>b. The perfusion bioreactor offers enhanced transport of nutrients, gases & metabolites due to effective media percolation through the interconnected pores of the scaffolds.<br>c. The modular design of the perfusion system facilitates multiple tissue-engineered construct production. | a. Though dynamic flow perfusion allows the media to percolate through the core of construct. The cells on construct surface experiences greater shear than the cells at inner core, creating a pressure gradient across the construct, which is typically not experienced by cells *in vivo*. |

actor system is also easily scalable and could be useful for large-scale culture of chondrocytes for clinical applications. Likewise concentric cylinder bioreactor is developed to culture tissue engineered cartilage under hydrodynamic loading conditions. When compared to spinner flask, concentric cylinder bioreactor operates at low shear stress and has a larger growth area for construct synthesis which ultimately creating more *in vivo* like environment for cartilage synthesis.

Though the above discussed reactors are successful in synthesis of few tissues types, mass transfer is one of the major concerns that have been an obstacle to produce thicker tissue construct *in vitro*. The flow perfusion reactor have promises to improve the quality of tissue constructs *in vitro* such as cell distribution and extra cellular matrix (ECM) deposition due to improved mass transfer with continuous flow of medium that increases convective transport. In flow perfusion system along with continues flow of media there is another variable play vital role is oxygenation of tissue constructs. Since oxygen is sparingly soluble in water, oxygen supply would be a limitation in larger tissue construct without vascular network. In this case bioreactors with flow perfusion system were used to improve oxygenation of thick tissue which ultimately yields high quality tissue constructs.

## CONCLUSION

Since the design of the first bioreactor, tissue engineering has reached greater stature. In this review, we

discussed the design and operation principles of various dynamic tissue engineering bioreactors for the cultivation of engineered tissues over 3D biodegradable scaffolds. When we monitor the construction of various reactors in detail we realize that specific design requirements depend on the dimensions, complexity and application of the tissue to be engineered. Since, physical and mechanical forces play crucial role in tissue development, designing of novel reactor to impart more sophisticated environment to cells and tissues *in vitro* offers significant options to improve functional tissue engineering constructs.

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