Development of bioengineering system for stem cell proliferation

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Abstract: From last decades, intensive research in the field of stem cells proliferation had been promoted due to the unique property of stem cells to self-renew themselves into multiples and has potential to replicate into an organ or tissues and so it’s highly demanding though challenging. Bioreactor, a mechanical device, works as a womb for stem cell proliferation by providing nutritious environment for the proper growth of stem cells. Various factors affecting stem cells growth are the bioreactor mechanism, feeding of continuous nutrients, healthy environment, etc., but it always remains a challenge for controlling biological parameters. The present paper unveils the design of mechanical device commonly known as bioreactor in tissues engineering and biotech field, use for proliferation of stem cells and imparts the proper growing condition for stem cells. This high functional bioreactor provides automation mixing of cell culture and stem cells. This design operates in conjunction with mechanism of reciprocating motion. Compare to commercial bioreactors, this proposed design is more convenient, easy to operate and less maintenance is required as bioreactor culture bag is made of polyethylene which is single use purpose. Development of this bioengineering system will be beneficial for better growth and expansion of stem cell

1. Introduction
Stem cells are ideologically undifferentiated candidates with their unique properties of Self-Renewal. These properties of stem cells are highly recognized to resolve many diseases such as spinal cord injuries, burns, liver cirrhosis, or failure of graft versus host disease, diabetes mellitus, progressive multiple sclerosis and cardiac ischemia are (diseases ) and etc., [1]. Due to unique characteristic of stem cells, it’s highly in demand but the actual number of stem cells can be obtained from available donors is very low for use as a tissue regenerative source, [2]. Successful implementation of stem cells based technologies are in highly needed of developing the system for high amount of stem cells with better density, [3]. Development of bio-engineering system is the promising system which results in the better expansion of the stem cells.

Bio-engineering system called as a Bioreactor is the closed vessel which provides the required biological parameters such as pH, Temperature, pressure, CO2, oxygen transfer and physiological parameters including nutrition. There are ample of the bioreactor systems available commercially like Hollow-fiber, T-flask, roller bottles and spinal flask. Each system is well defined with their results but still there are some limitations such as growing cells in micro-carriers, like sterile bioreactor may generate large shear forces which dislodge the stem cells from the micro-carriers and it may even cause the damage to the stem cells. This mechanism is commercially available and inexpensive. Other
excellent devices such as hollow-fiber bioreactor and fluidized bed bioreactors have turned out more complexes to replace the spinner flask for cell cultivation, [4]. Bioreactor systems target a reliable and efficient procedure, enabling highly reproducible, automated and controlled process which is compatible with good manufacturing practice (GMP) requirements, [5, 6]. Development of new bioengineering system with new principle will give rise to new-era in the field of Bio-Tech engineering, where the collaborative studies of bio-medical and engineering will demand more research outcomes in future.

2. Proposed Design

The paper unveils the reciprocating motion based mechanism with donut shape culture bag which will be single use purpose. This Bioreactor consists of basically three Units. First one is chassis of bioreactor, second is bioreactor platform and third and donut shape culture bag. These three parameters play important role in whole system. Detail description has been given as below.

2.1.1. Design Structure

In proposed design, Circular disc shaped actuator is mounts on the shaft of the electrical motor which transmits the electrical energy of the motor to the circular motion. The two parallel studs are attached vertically on the circular disc-shaped actuator which will restrict the motion of angular arm and T-Pin. As shown in figure, angular arm is mounted on roller bearing which is fixed with the solid based. The T-pin is mounted on the U-Shaped solid bar with platform supporter. The motion of the U-Shaped bar is reciprocating where bar is guided by the rollers which help to convert the circular motion to the reciprocating motion. The supporter of the platform is attached with two bearings bearing at the bottom of the system to give support of the rod which gives load to electrical motor and due to higher load torque will increase and rotation of motor decrease indirectly. This helps us to maintain our rpm. The main purpose of this design is to produce as less as possible sheer force and bubble generation and also provide smooth rotation for better quality of growth and large proliferation of any kind of stem cell.

2.1.2. Donut shaped Single use Bioreactor Bag

The single use disposable bag is pre-sterilized and sealed which plays big role in expansion of stem cells. The disposable bag is made of plastic material like polyvinyl chloride or polymer approved by human medical use standards. The bag has a donut shape with central hole, which is place on the design platform. For shape and rigidity, clean filtered air is filled in the bag.
The inlet and outlet tube of the bag is sealed with silicon plug and cap. A pre-sterilized single use disposable bag is placed on the platform, partially filled with the media. It is inflated afterward by using integral sterile inlet filter method.

![Donut shaped SuB culture bag with inlet and outlet tube](image)

**Figure 2.** Donut shaped SuB culture bag with inlet and outlet tube

### 3. Biological Parameters

For this proposed design, we consider some physical and biological parameters are, mixing ratio, expansion of stem cells, cells density, and oxygen transfer and time distribution which plays big role for proliferation of stem cells.

#### 3.1.1. Stem cell culture

For different type of the stem cells there are different types of culture media and nutrition required. With the help of the medical team with choose Mesenchymal stem cells to get clear idea about environment and parameters required. Human MSCs were isolated from the umbilical cords and by surface marker analysis with functional characteristics; the Isolate stem cells are announced as a mesenchymal stem cells [7]. Experiments are performed with cells passages 3 to 6. Stem cells are cultivated in αMEM containing 1 g/L glucose, 10 % human serum and 50 µg/ml in a humidified atmosphere containing 5.5 % CO₂, 23 % O₂ at 36 °C.

#### 3.1.2. Time Distribution

For better mixing in the bioreactor, the residence time destruction is the one of the technique used widely [8]. To determine the residence time, direct pulse is used which are predicted by adding the methylene blue, substance in the bioreactor. This tracer substance is injected by inlet tube. By adding methylene blue, the Dirac pulse is predicted and the tracer substance in to the single use bioreactor in 5-10 s of time.

#### 3.1.3. Expansion of the stem cells in the reciprocating motion based bioreactor

Already preserved UC-Mas are been used for expansion of the MSCs in the Bioreactor. The bag is seeded with cell density of 1200 cells/Cm² once the environment of the bag is stabled. Firstly these cells are seeded in the incubator for one day then bioreactor is filled with cell suspension with MSCs over two passages in in αMEM supplemented with 10 % human serum and 0.5 % gentamicin at 37°C. Afterwards, the motion of the bioreactor stars with 6-16 cycle/min while feeding rate and MSCs culture media are ready to mix with stem cells. As per our predictions, each day growth of the cells are depend on glucose consumption and lactate production.

### 4. Result and Discussion

#### 4.1.1. Oxygen Transfer

The reciprocating motion bioreactor bag has gained increasing mass transfer than the culture static we used slope of mass balance equation to calculate the K_L a which is...
\[ k_{aL}(t_2-t_1) = \ln \left( \frac{C^* - C_1}{C^* - C_2} \right) \]  

(1)

where: \( C^* \) = the saturation DO; \( C_1 \) = DO at time \( t_1 \) and \( C_2 \) = DO at time \( t_2 \).

To analyse the result, he oxygen transfer is obtained from the spinner flask from available report named Aunin et al.(1989). According to that report, \( K_{aL} \) of a 1 l spinner with 500 ml was counted 1 hr\(^{-1}\) and Dorrestenijn et al. 91994) value of \( K_{aL} \) in 600 ml spinner system with 300 ml media was around 2hr\(^{-1}\). When the oxygen transfer rate into 1000 ml of media with result a \( K_{aL} \) of 3 hr\(^{-1}\) is compared with \( K_{aL} \) for 1000 ml of Media in 3 L spinner, three-fold higher results are derived. This could be potential result in three fold higher maximal cell density in the reciprocating motion based bioreactor.

4.1.2. Mixing ratio

Rpm plays one of the important roles to mix the cells and culture media. Under different situations, culture media and cell mixing time are approximated by videotaping and injecting a fluorescent. In the reciprocating motion we can predict better mass transfer rate with changing the air flow and rpm.

4.1.3. Biological Parameters

After the cultivation, the expansion of the MSCs in the reciprocating motion based bioreactor is maximized over 14 days. The total expansion of the stem cell number is 23.5 ± 2.6x10^6 and 8.2 ± 0.7 fold. The growth rate is increased by 8 fold after 14 days. Throw-out this period of time DO is controlled by at least 42 % as well as pH and CO2 is regulated automatically.

5. Conclusion

The SuB with round shape bag has a concept of newly proposed design of reciprocating motion based platform which also can be used for different single use culture bag. The main goal is to develop new design of SuB which is self-contained and completely mobile with great potential and different mechanism which prevent the clogging of the filters which are already placed inside the Single use bag. The mixing ratio and oxygen transfer is maintained with controlled speed and motion direction. The function of the SuB results in increased size and better quantity of stem cells. Till now oxygen transfer, results in three fold higher maximal cell density in the reciprocating motion. Still deep knowledge is needed to select the all physical parameters exactly which can give rise to sell proliferation. Once we acquire the deep knowledge, it will be easily possible to reflect better results. Future challenges include the development of clinical and laboratory scale bioprocess system. As we already aware about different parameter for each stem cell we have to develop the sustainable and effective system for better proliferation of stem cells. We also want to realize the effect of different shear force, rpm and circular motion with different biological parameter.

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