DESIGN OF A PERFUSION BIOREACTOR FOR SIMULATING SYNOVIAL JOINT CAVITY

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CERTIFICATE

This is to certify that the thesis entitled, "**DESIGN OF A PERFUSION BIOREACTOR FOR SIMULATING SYNOVIAL JOINT CAVITY**" submitted by **AMRIT PRAHARAJ** in partial fulfilments for the requirements for the award of Bachelor of Technology Degree in Bio-Medical Engineering at National Institute of Technology, Rourkela (Deemed University) is an authentic work carried out by them under my supervision and guidance. To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University / Institute for the award of any Degree or Diploma.

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ABSTRACT :

Enthesis is the point of attachment between the bone with a tendon or a ligament. It seems more assuring to use a tissue engineered graft that is installed with fibro cartilage which is regenerated by the help of stem cells differentiation than to use a ligament alone graft to replace an injured ligament. Hence a bioreactor system is necessary to mimic this concept and environment of enthesis. A bioreactor system is planned that can be used to conduct perfusion of a cell seeded scaffold for a ligament graft with a medium similar to synovial fluid composition. Various parameters like pressure, temperature, oxygen, carbon dioxide, PH etc. in the bioreactor can be controlled and regulated to simulate the *in-vivo* conditions. The bioreactor could also provide a two dimensional movement that would impart a compressional and tensile force to the scaffold for development of enthesis. Thus it would mimic the in vivo conditions of a joint cavity and simulate the environmental condition which is necessary for the development of enthesis.

(keywords : bioreactor, enthesis, scaffold,movement)

CHAPTER 1

INTRODUCTION

1. INTRODUCTION :

A Bioreactor can be any device which is used to artificially produce a biological environment in-vitro or which is capable of growth of organisms such as bacteria or fungi in a biological environment under controlled conditions. It can be used in varied applications such as in aerobic/anaerobic chemical processes, in fermentation, in cell culture applications, to mimic the working of organs such as kidney, heart as thus behaving as artificial organs, tissue engineering applications, in industrial applications such as production of antibodies, vaccines, in bio-conversion for producing by-products from raw materials etc [1] .The environment within a bioreactor is maintained and controlled in such a manner that constant homogeneous conditions are maintained . The figure below shows a typical commercial bioreactor:

FIGURE 1

CHAPTER 2

BIOREACTOR: TYPES AND COMPONENTS

2.1 Types of Bioreactor :

Based on the functioning of the Bioreactor it can be of 3 types:

1. Batch Bioreactor
2. Fed-Batch Bioreactor
3. Continuous Bioreactor

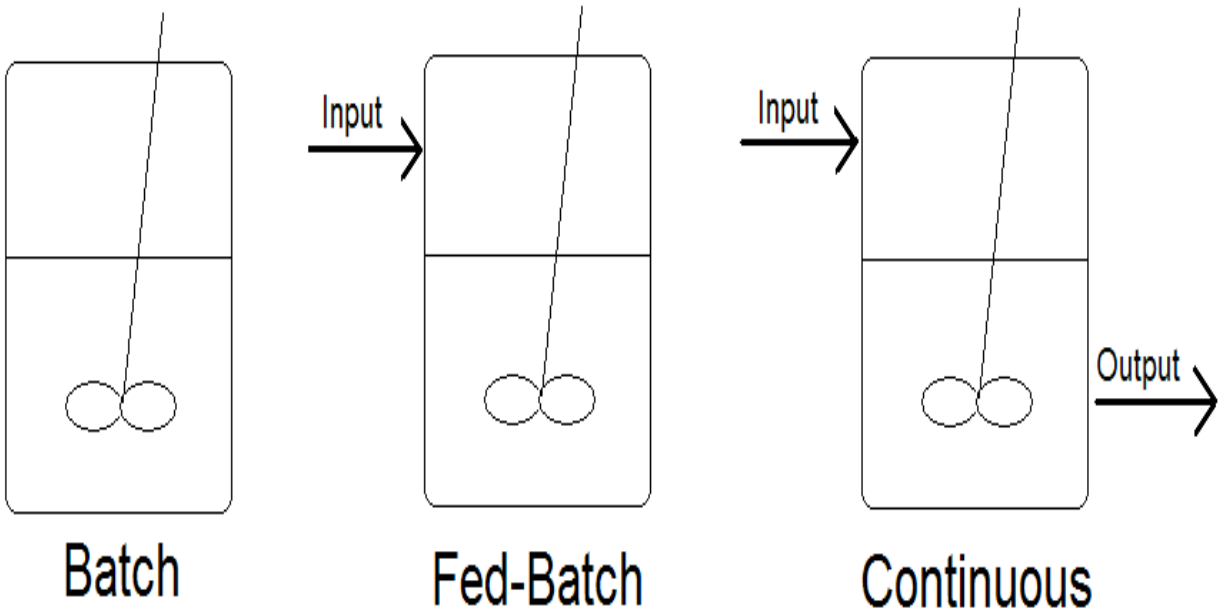


FIGURE 2

Batch Bioreactor: This bioreactor's volume is constant and cells and media are added for performing the operation in batches. The operation is run until a particular set point in terms of time and concentration is reached. Then the used media is drained out to fill it with a fresh new media.

Fed-Batch Bioreactor: In the beginning it starts as a batch process. After reaching a particular point, a input is given .Here the volume of the bioreactor increases after the input is provided.

Continuous Bioreactor: Here there is a continuous flow of media solution containing cells from input end to the bioreactor. The used solution and product is also taken out continuously from the bioreactor. The rate of input and output is maintained in such a manner that the volume of the bioreactor is constant.

Among all the bioreactors, continuous bioreactor has the advantage that it is automated as compared to batch and fed-batch bioreactor where every time after batch the operation has to be started again. Hence it requires less manpower and the productivity is more. [2]

2.2 COMPONENTS OF BIOREACTOR :

Bioreactor can come in any size and configuration. A typical bioreactor has got many components such as:

- Vessel or Chamber
- Motor agitator/Impeller
- Gas Sparger
- Temperature sensor
- PH sensor
- Pressure sensor and valves
- Flow meter
- Cooling jacket

- Input and Output port
- Filters

VESSEL/CHAMBER :

The vessel/chamber is the main housing where all these components will be loaded and the media solution containing the cells will be placed. The vessel should be biocompatible so that it can be autoclaved to remain contamination free. Hence the material used can be stainless steel or glass. Glass can be used as it is transparent and can give a clear view of the working of the components inside.

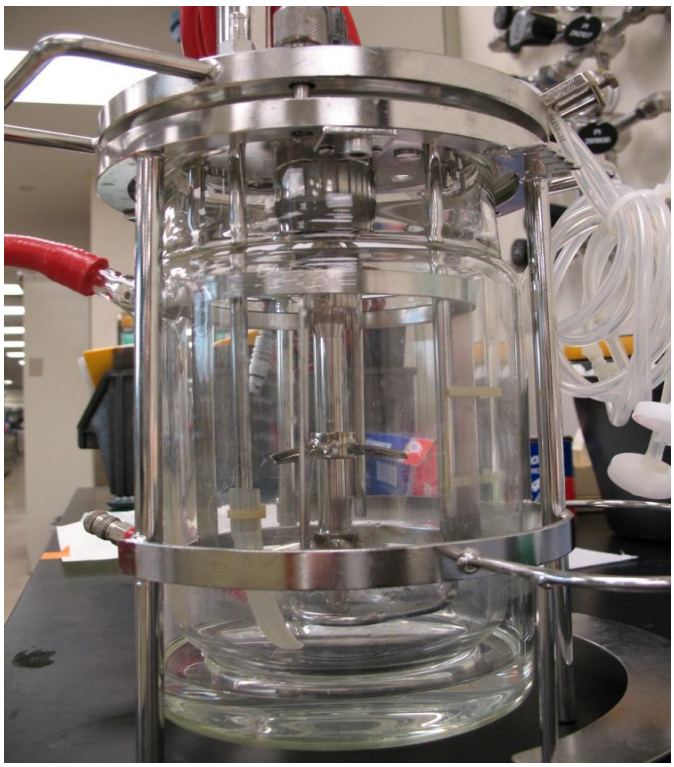


FIGURE 3

MOTOR AGITATOR/IMPELLER:

An agitator is used so as to mix all the contents in the bioreactor so that the solution contains all the nutrients, gases in equal proportions and homogeneous conditions with constant PH and temperature.

AIR SPARGER:

The Sparger helps to introduce and mix gases into the solution. It maintains the level of oxygen and other gases required for the bioreactor system.

SENSORS:

Different sensors and valves to monitor the conditions of the bioreactor are used such as temperature sensors, PH sensors, pressure sensors, flow meter etc.

COOLING JACKET:

The cooling helps to reduce and maintain the temperature by flow of a coolant around the bioreactor.

VALVES, FILTERS, PORTS:

Different ports are used to connect various components in the bioreactor system. Various filters can be used in a bioreactor to either filter the gases entering or leaving the system or filter the media solution leaving the system to maintain the PH and keep it constant. Various valves can be used to control the flow of gases and liquid solutions. [3]

CHAPTER 3

BASICS OF PERFUSION BIOREACTOR, SYNOVIAL JOINT.

3.1 SYNOVIAL JOINT CAVITY :

Synovial joint is a movable joint commonly found in any mammal .The capsules around the articulating surfaces and the synovial fluid in the synovial cavities which are present in the capsules makes it different from fibrous joints. Synovial joint contains structures like articular capsule, articular cartilage, synovial cavity etc.

Synovial cavity contains synovial fluid which helps in lubrication and nourishment of the cartilage. Articular cartilage helps in the movement of the joint by providing a surface that has low friction coefficient and hence helps to reduce the friction by absorbing shock thereby providing a smooth movement. Synovial joints allow various movements like Abduction, Adduction, Flexion, Extension and Rotation. Some of the common synovial joints are Ball and Socket joint, Hinge joints, Saddle joints, Pivot joints, Condyloid joints, Gliding joints etc.

Stability of the synovial joint is affected by various factors such as:

1) Articular surfaces shape
2) Structures like capsules, ligament
3) Tone of the muscle
4) Gravitational forces
5) Pressure(sub-atmospheric) [4] .

3.2 PERFUSION BIOREACTOR :

INITIAL APPROACHES

Before the use of perfusion bioreactors various bioreactors of different designs were used. The most primitive method for cell culture and tissue engineering applications was independent cell seeding on three dimensional scaffolds. Some methods that followed this included spinner flask method and rotating wall vessel reactor method. In the spinner flask method scaffolds seeded with cells were allowed to be attached and hanged in the flask. The fluid solution was allowed to move through it with the help of a magnetic stirrer.

In the rotating flask method the bioreactor consisted of two cylinders. The annular space between them was the space where the scaffold was suspended. The inner cylinder was permeable where gas exchange occurred while the outer one was impenetrable and rotated in such a manner that the centrifugal forces balanced the gravitational forces to keep the scaffold suspended.[5]

PERFUSION BIOREACTOR:

To increase the rate of mass transfer through the three dimensional scaffolds perfusion bioreactors was used. Using a pump a continuous flow of media solution was produced which was allowed to perfuse through the porous scaffold. The flow of the fluid should be maintained in such a manner that the fluid enters through the scaffold. The chamber in which this perfusion of the fluid takes place is called perfusion chamber. As the media flows through the porous structure of the scaffold the mass transfer is enhanced as shown in the picture below.[5]

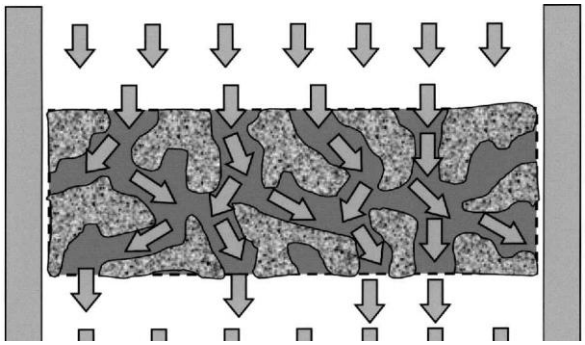


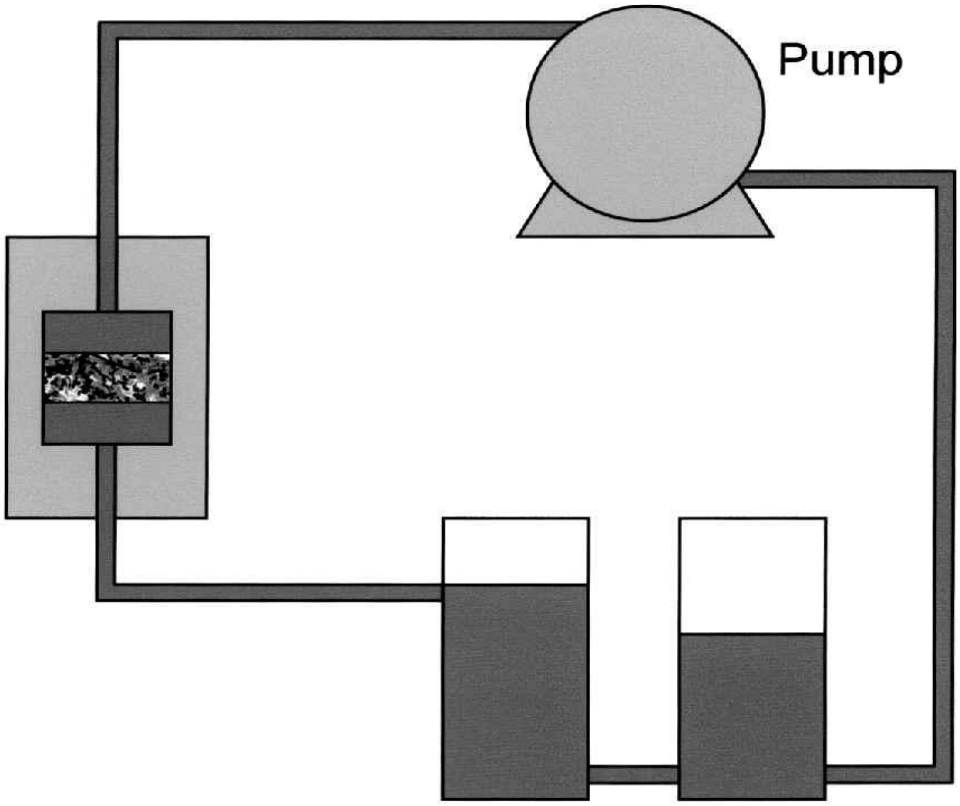
FIGURE 4

Source : Gregory N.Bancroft et.al ,Design of a flow perfusion bioreactor system for bone tissue engineering applications.

A flow perfusion bioreactor must have the following things in its design:

1. It must allow the fluid to flow through the scaffold rather than around the scaffold .This would minimise the loss of perfusion in the scaffold and hence would be better than the previous bioreactor designs discussed as spinner flask and rotating wall vessel reactor method.
2. The flow should be continuous and repeated throughout the time of the operation. It should be such that it can be controlled and can be made consistent to flow through the scaffold.
3. To avoid contamination problems and errors induced due to it should be sterilised and autoclaved throughout the time of operation.
4. The system should be simple and easy to operate. The more complex the system becomes, more chances of induction of errors due to different factors come into play.[5]

A typical perfusion bioreactor is shown below



Source : Gregory N.Bancroft et.al ,Design of a flow perfusion bioreactor system for bone tissue engineering applications.

FIGURE 5

This design contains one pump on the top, one chamber shown on the left hand side of the diagram and two medium reservoirs on the bottom side of the diagram. The individual scaffolds are placed in a cassette system which will allow the media solution to perfuse through each scaffold. The flow of media solution is maintained using the pump shown. The

media solution flows from the chamber to the first reservoir which then goes to the second where it is pumped back and perfused through the chamber. When the media solution has to be changed the connection between the two reservoirs is closed. The used media solution from the first reservoir is removed; it is then washed and again filled with fresh media solution.

Advantages of perfusion bioreactors over other bioreactors:

1. The operation is easy and automated; hence less man-power is required.
2. As the operation is continuous and consistent, the productivity is more.
3. It provides a closed system which is contamination free and is safe.
4. The flow of media solution and the fluid pathways mimics the flow of in vivo interstitial tissues. The media flow can be controlled and maintained according to the needs of the experiment
5. The efficiency and the accuracy of this system is more as more than one experiment can be run simultaneously within the system [6].

In this project a perfusion bioreactor system is to be designed to simulate the working conditions of a synovial joint cavity which would be able to mimic and simulate the environmental conditions of a joint cavity in-vitro. The bioreactor system would be made such that the media solution composition would be similar to synovial fluid with a pressure range of sub-atmospheric to zero pressure. Thus it would mimic the flexion and extension movement of the synovial joint. It would help in the growth of a graft made in the order of bone-ligament-bone (B-L-B).This graft would be pre-installed with interfacial (bone to ligament interface) fibrocartilage called enthesis. This hybrid graft would comprise a multi-compartmental tri-lineage coculture which would be coming out of this developed bioreactor

system. This would remove the very common problem of decreased insertional strength at bone-ligament junction that occurs in ligament alone grafts which are in developmental stage or in the current surgical procedures which are done to replace damaged ligament (i.e. grafts such as Patellar tendon graft and Hamstring graft for Anterior Cruciate Ligament replacement). The bioreactor chamber housing would comprise of the following parts:

1) A moving mechanism 1 that would allow to compress the stem cell compartment which would ultimately differentiate into enthesis
2) A moving mechanism 2 that would allow to stretch the ligament compartment that are present between the bone compartments keeping the stem cell compartments at the junction
3) The scaffold would be placed in the cassettes system.

. The cassettes also provides a two dimensional movement as mentioned above as moving mechanism 1 and moving mechanism 2 to the scaffold. The moving mechanism 1 would be the movement in the vertical plane that would compress the scaffold at the proposed location. The moving mechanism 2 would be the movement in the horizontal plane which is used to provide the tensile force to the scaffold.

CHAPTER 4

MATERIALS AND METHODOLOGY

All the models described have been designed using SolidWorks 2009 SP0.0 software. SolidWorks is mechanical modelling software which helps to design any three dimensional model and simulate the mechanical moving parts of the model in a complex system.

4.1 CHAMBER:

The chamber is the main housing that holds all the components inside it. The chamber is made transparent for easy access and clear view of the operation that goes inside it. The three dimensional chamber model is designed in SOLIDWORKS .The following dimensions has been set in the design which can be changed or modified. The length has been set to 270.2 mm, breadth as 221.28 mm and the height as 262.126 mm. The chamber roof has got five rods which is coming from the top of the roof and enter into it. The rods are made up of 4 mm diameter. Four rods enter into the chamber at four corner points of the chamber while one rod enters in the centre of the chamber. The rods would connect the in housing to provide compressional and tensile force from outside.

The two directional movement can be done by applying moving mechanism 1 and moving mechanism 2 i.e. movement in horizontal plane at the same time single movement can also be applied independent of the other.

4.2 CASSETTE SYSTEM :

The cassette system is mainly designed such that it can support and hold the hybrid scaffold comprising of trilineage coculture .The three dimensional design is done is SOLIDWORKS software. The cassette system consists of two cassettes: left cassette and right cassette which are symmetrical in nature. Each cassette has got two parts: lower part and upper part. The lower part of each cassette is being attached with three pillar structures. The lower structure must also have four locations for holding four scaffolds. Now the upper part of each cassette is made in such a way that it can move in vertical plane i.e. moving mechanism 1 and can provide a compression force to the scaffold. The upper part has got a structure which has four protruding bars coming out of it. As the upper part moves in the vertical plane in moving mechanism 1 these bars compress the scaffold. The protruding bars have been given the following dimension in the design: length is given as 40 mm, width and height is kept as 20 mm each.

The pillar structures that is attached with the lower part of each cassette is responsible for creating the movement in the horizontal plane thereby creating moving mechanism 2.The lower part of each cassette is attached with three pillars : one at each corner at one at the middle. The pillar structure is designed is such a way that it begins with a square surface structure which has got a tapering end and it ends with a spherical roller structure. The spherical rollers at the end of each of these three pillars help in the movement in the horizontal plane i.e. moving mechanism 1.The square surface has got a dimension of 8 mm edge and the spherical roller has got a radius of 7 mm. The length of the cassette is kept 170 mm, width as 50 mm and height as 10 mm. A spring system is attached in the cassette system that assists in moving mechanism 1 and moving mechanism 2.

4.3 BASE:

The base of the platform is the place where the cassette system fits and moves in the moving mechanism 2 in horizontal plane. The base has got three grooves for the three pillars of each cassette to fit in .Hence in total there are six grooves in the base. The grooves are spaced in a symmetrical manner for both the left and the right cassette. For each cassette the grooves are spaced in such a manner that the length between the outer edges of the extreme grooves is the length of each cassette. To provide support to each of the three pillar structure each groove is made such that the spherical roller can be placed in the groove first and then can be slided into a cylindrical groove (within the main groove) where the spherical roller of each pillar completely fits and hence can move forward or backward for the moving mechanism 2 to occur. As the spherical roller of each pillar completely fits in the cylindrical groove within the main groove of the base, the pillar is very stable and hence there is no chance of falling down of the cassette system. For complete fitting of the spherical roller of each pillar and the cylindrical groove, the diameter of both the spherical roller and the cylindrical groove must be equal.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 RESULTS:

All the designs were made in SOLIDWORKS software. The designs were made component wise and were finally assembled to make up the complete system. The designs are shown in the order as listed below from the next page onwards.

1) chamber housing
2) base
3) groove (within the base)
4) pillar structure
5) pillar structure in groove
6) cassette
    - upper part
    - lower part
7) Base and cassette system merged

Fig 6 to Fig 11 below shows the designs of the individual components in an animated oblique view.The blue prints of the cassette system and the base are given in the appendix on which the fabrication is being done.

BASE:

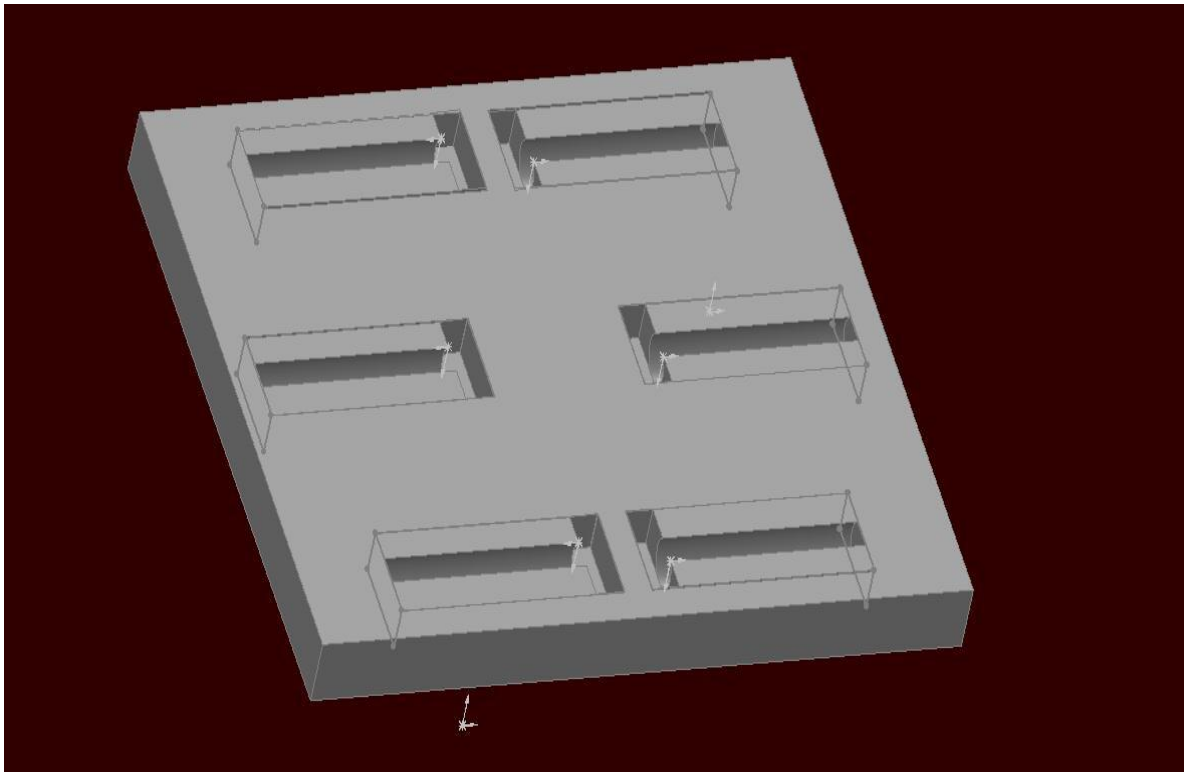


FIG 6: Base

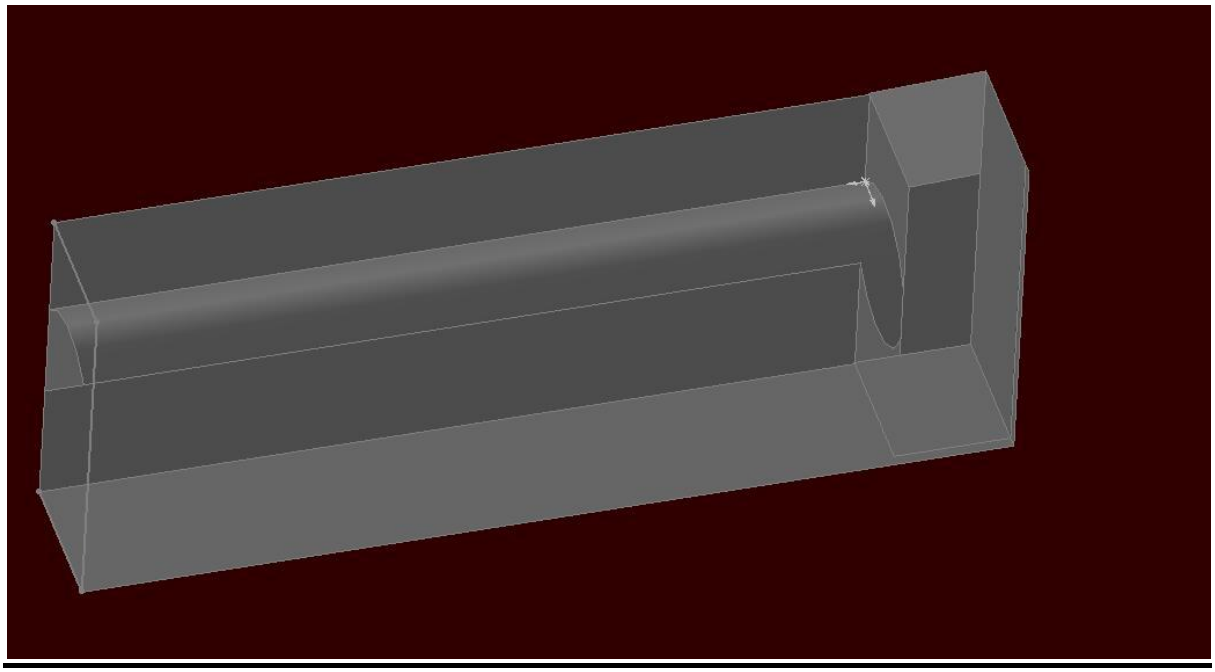


FIG 7: Groove (within base)

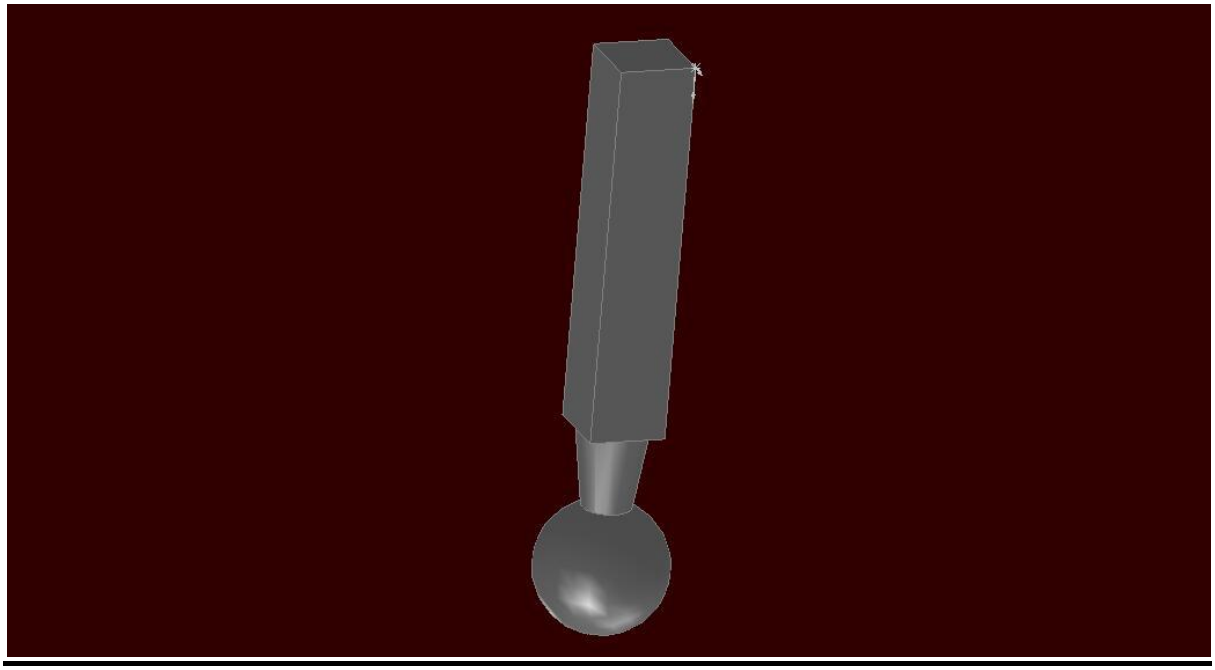


FIG 8: Pillar Structure

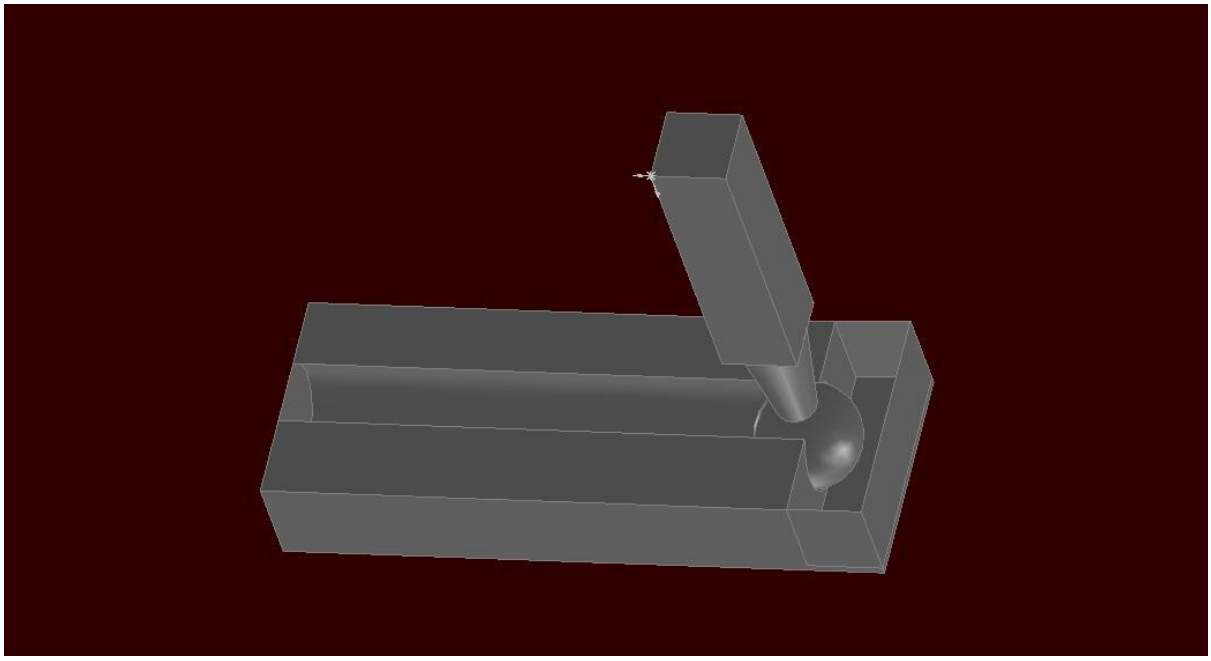


FIG 9: Pillar in groove

CASSETTE SYSTEM:

ONE HALF OF UPPER and LOWER CASSETTE PAIR:

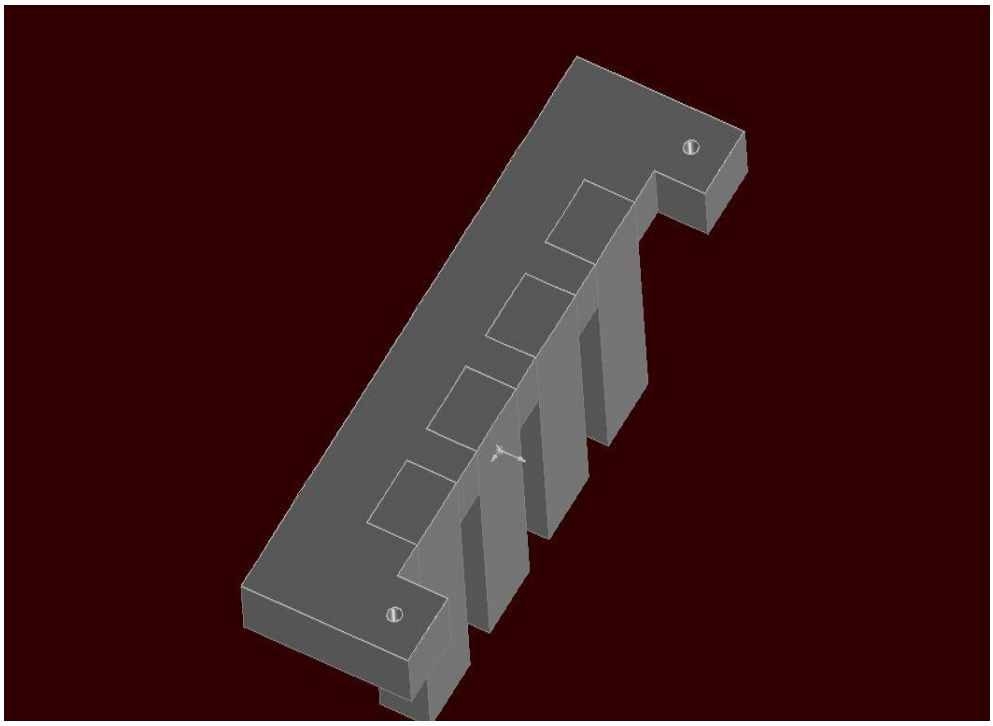


FIG 10: one half of upper cassette pair

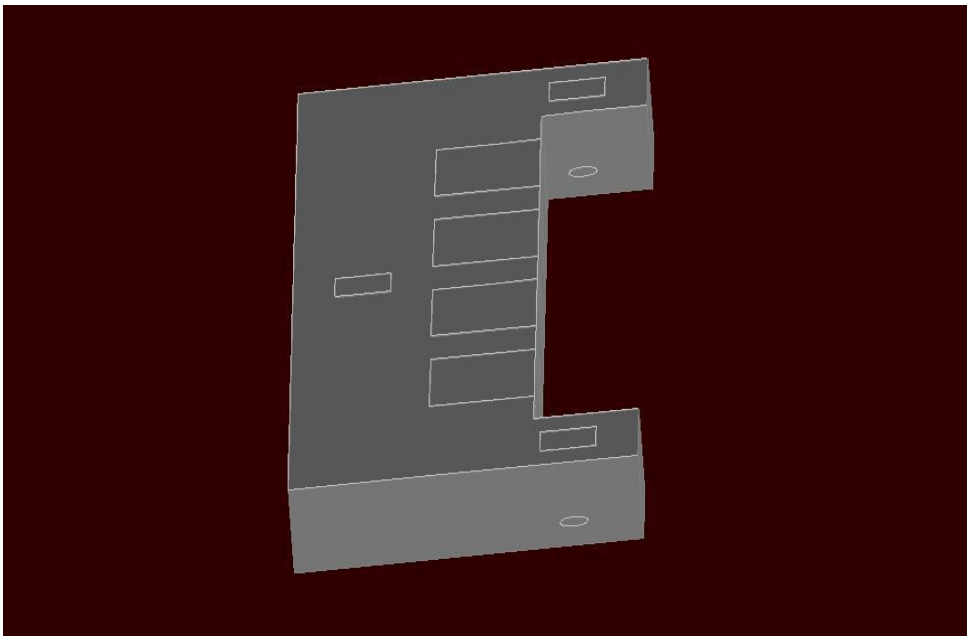


FIG 11: one half of lower cassette pair

5.2 DISCUSSIONS:

Thus the whole bioreactor system has been designed such that this hybrid graft would remove the decreased insertional problem which is a very common problem with ligament alone grafts which are in developmental stage or the present surgical procedures which aim at replacing completely damaged ligament. A bone-ligament-bone graft can replace a ligament which is injured partially or completely. The present ligament alone grafts which are being developed in laboratories does not have the insertional strength after implantation which takes place through a number of surgical interventions. The reason for this is that it does not cover the regeneration of enthesis which is found in vivo anatomically at the junctions of bone and ligament. In neonates the enthesis growth is dependent on the interactions that take place between the cells. This process is followed up by stimulus most likely compressional force later in life which helps to maintain the enthesis development.

CHAPTER 6

FUTURE WORK

6.1 FUTURE WORK:

The future work would include the completion of the fabrication process and characterisation of the bioreactor. The bioreactor which would be developed in our laboratory would simulate the mechanical moving conditions and hence would fasten up the process of the growth of enthesis. The bioreactor would provide a two dimensional movement i.e. a compressional force at the junction of bone and ligament and a tensile force at the ligament part of the scaffold. The bioreactor system would be made such that the media solution composition would be similar to synovial fluid with a pressure range of sub-atmospheric to zero pressure. Thus it would mimic the flexion and extension movement of the synovial joint. The scaffold that would be used in the system would be a three dimensional polymeric scaffold which would be seeded with human mesenchymal stem cells with a trilineage co culture.

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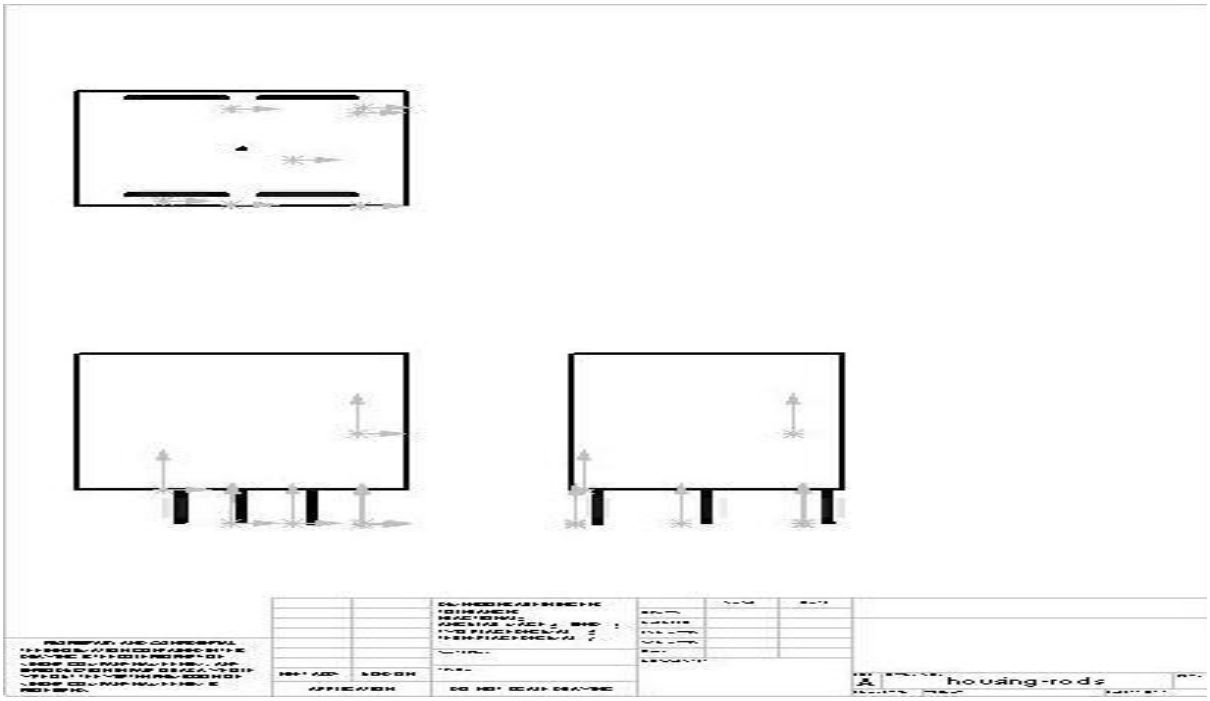
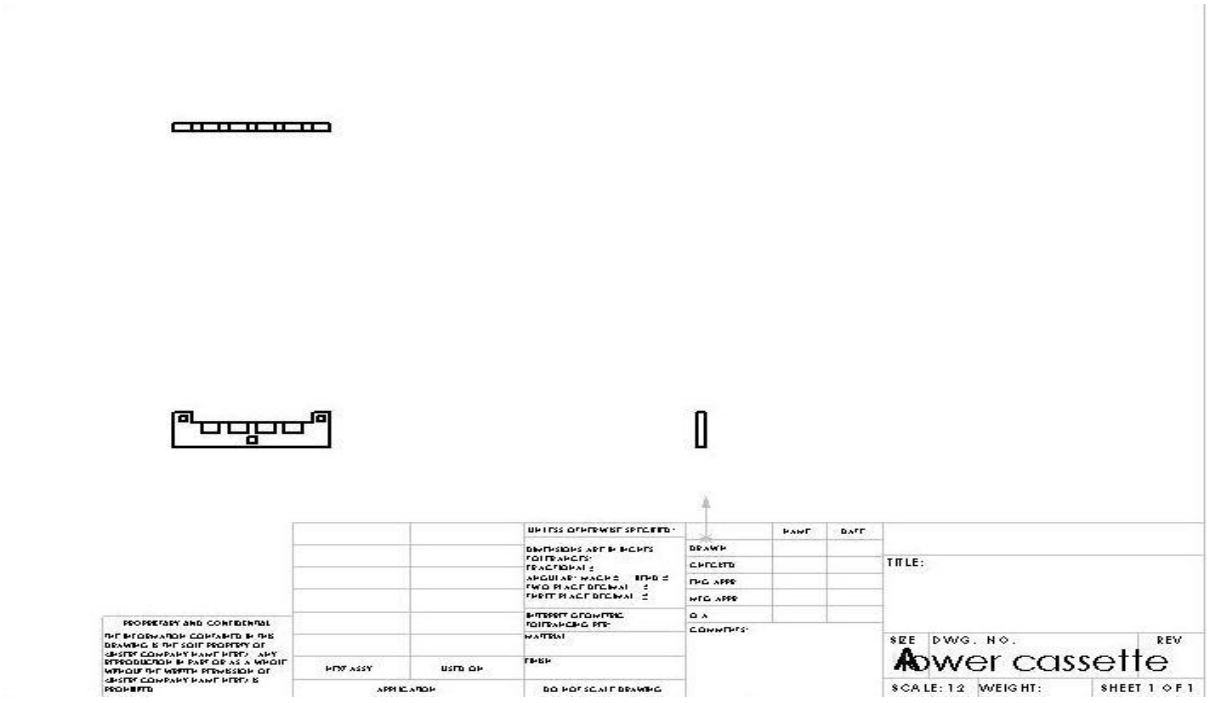
APPENDIX

Blue print of Upper Cassette:



Blue print of Lower Cassette



Blue print of the base