MECHANOBIOLOGIC RESEARCH IN A MICROGRAVITY ENVIRONMENT BIOREACTOR

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Abstract

The aim of the present research is to prepare a background knowledge in the field of bioreactors especially those for use in the microgravity environment. The work is addressed to two different audiences, engineers and biotechnologists. The paper deals with two main aspects of the design of a bioreactor: the biological aspects of a culture of cells, a critical concept in the design of a bioreactor, and its technical characteristic. The biological part attempts to give a short overview of the different problems arising during cell culturing, with special attention given to mammalian cells. In the technical part, the ways in which different devices obtain different characteristics of the culturing environment are described and analyzed with special attention to the simulated microgravity environment obtained through the technique of time averaging of the weight vector.

Introduction

In the past decades, fundamental advances in cellular and molecular biology have improved the ability to culture cells in vitro. Cells and tissue cultures are a major area of research for biotechnology community. Tissue culturing is one of the basic tools of medical research and is a key to developing future medical technologies such as therapeutic trial prior to in vivo experimentation and tissue transplantation. A current problem in tissue culturing technology is the unavailability of an effective bioreactor for the in vitro cultivation of cells and explants. It has, in fact, proved extremely difficult to promote the high-density three-dimensional in vitro growth of human tissues that have been removed from the body and deprived of their normal in vivo vascular sources of nutrients and gas exchange. Nowadays advances in scientific knowledge and biotechnological capabilities herald an exciting new betrothal between tissue engineering and space biology.

In this alliance, microgravity may become a surprising, unconventional, and yet attractive venue for the generation of macroscopic tissue. The physical basis for these effects is thought to be due to the low-shear fluid environment that cells and tissues encounter in real or simulated microgravity. By eliminating sedimentation, buoyancy and density driven convection, cells can be grown in a relatively quiescent fluid environment, where interactions between cells can occur with minimal disruption. This would allow cell aggregates and tissues to form as in vivo, but also suggests that methods are required, by which the mechanical environment may be modified to control the development of engineered cell structures. The environment created on Earth within a clinostat or rotating vessel is often referred to as "simulated microgravity". Presently, we know that microweight does evoke a number of effects on cells, and a number of well-controlled experiments have shown that the absence of weight can have profound effects on fundamental biological processes active within the cell.

Cells culture

Each tissue in the body is composed of dynamic assemblage of multiple stationary and migrating cell types that are embedded in a complex macromolecular structure. For proper cell propagation, the cell culturist needs to provide an in vitro environment as similar as possible to the natural cell environment (e.g. uniform conditions, transport nutrients, oxygenation, hydrodynamic...
stresses, etc.). Such an environment is provided by a device known as 'bioreactor'. Most commonly, cell culture is performed in two-dimensions, such as in petri dishes, tissue culture flasks, multi-well. In spite of the tremendous amount of information gained in traditional cell culture settings, it is generally acknowledged that conventional tissue culture in two dimensions may be inadequate to model the complex cellular interactions that promote tissue-specific differentiation as they occur, e.g. during organogenesis. A variety of tissue explants can be maintained for a short period of time on a supportive collagen matrix surrounded by culture medium. But this system provides only limited mass transfer of nutrients and wastes through the tissue, and gravity-induced sedimentation prevents complete three-dimensional cell-cell and cell-matrix interactions. Culture longer than 7 week have been difficult to achieve, since crypt cells are unable to survive standard culture regimens. As the scale of cultivation increases, mixing of the culture medium is required to maintain uniform conditions and enhance the transport of limiting nutrients such as oxygen. Since mixing is accomplished by deformation of fluid elements, hydrodynamic stresses are transmitted to cells. Several devices presently on the market have been used with only limited success since each has limitations which restrict usefulness and versatility. Further, no bioreactor or culture vessel is known that will allow for unimpeded growth of three dimensional tissue or organs.

Most of the attempts to culture tissue-engineered constructs in vitro have utilized either stationary cultures or systems generating relatively small mechanical forces. For example, cartilage constructs have been cultured in spinner flasks under mixed or unmixed conditions, in simulated and in real microgravity. In these mixing studies, however, it is difficult to definitively quantify the effects of mixing-induced mechanical forces from those of convection-enhanced transport of nutrients to and of catabolites away from the cells.

Mammalian Cells Culturing
Mammalian tissue can be grouped into three general categories: organ tissue, structural tissue and blood producing tissue. Mammalian tissue is composed of aggregates of cells that share a functional interrelationship in order to have tissue growth. Mammalian tissue is composed of different types of cells characterised by different morphology and immunochemical properties. Soluble signals produced and shared among the cells play an important role. Cellular differentiation may depend on three simultaneous conditions:
- Three-dimensionality
- Low shear stress and turbulence
- Co-spatial arrangement of different cell types and substrates

The composition of the ECM is variable depending on the type of tissue and its stage of development, and some of the components may undergo a transient change in distribution in response to environmental stimuli or disease states. It mostly consists of polymerised collagens, structural glycoproteins, elastin, glycosaminoglycans, adhesive laminin, fibronectin, arranged in a complex mesh that is constantly bathed by the fluid of the interstitial tissue space. The ECM is also known to be critical for regulating cell morphology, proliferation and differentiation, and is capable of responding to various endocenous and exogenous stimuli.

Mammalian cell culture and tissue generation, is much more complex because such cells are more delicate and have a more complex nutrient requirement for development. High-density, three-dimensional in vitro growth of human cells is problematic due to turbulence and shear effects, or inadequate oxygenation in conventional cell culture systems. In addition, mammalian cells can have a special requirement because most animal cells must attach themselves to some surface in order to duplicate. These cells are addressed as anchorage-dependent mammalian cells that have thin cell membranes. To provide the necessary attachment surface with a large surface area/volume ratio, small "microcarrier" (or beads) are suspended in fluid medium with fresh nutrients. Anchorage-dependent cells have been widely cultured on microcarriers. After several division, cells can form a confluent monolayer, with 100 to 200 cells on each microcarrier. Many cell covered microcarrier attach and bridge to form larger cell assemblages. Cell assemblages have been grown up to several millimetre in diameter.

The problem then is to suspend microcarrier without inducing turbulence or shear forces which will damage cells. Unless a cell culture is growing in an environment free of gravitational forces, moderate levels of agitation are required to suspend microcarriers that are not neutrally buoyant. Most conventional bioreactor for microcarrier culture have used internal propellers or movable mechanical agitation devices which are motor driven so that the moving parts within a vessel cause agitation in the fluid medium for the suspension of mammalian cells carried on beads. However, this agitation induce fluid turbulence and
shear. Mammalian cells cannot withstand excessive turbulent action without damage to the cells and must be provided with a complex nutrient medium to support growth. Small bioreactor vessels with internal moving parts may damage mammalian cells and also subject the cells to high fluid shearing stresses. If the beads collide one other in the suspension, the attached culture cells can be damaged. Therefore, bioreactors that utilise mechanical parts, air or fluid movement as a lift mechanism to achieve particle suspension will likewise cause damage to growing cells and tissues due to fluid shear.

**Microgravity and Simulated Microgravity**

Normal mammalian tissue and the culturing process has been developed for the three groups of organ, structural and blood tissue. The cells are grown in vitro under microgravity culture conditions and form three dimensional cells aggregates with normal cell function. The microgravity culture conditions may be microgravity or simulated microgravity. Simulation of microweight under normal ground conditions is usually based on the 'requirement' that the weight vector should act for at least seconds in a constant direction to generate an effect on cells. Conditions in which this requirement is met actually prevent the cell from feeling weight at all; the weight vector escapes its detection machinery. The machines described are based on the hypothesis that sensing no weight would have similar effects as being weightless. One approach is to provide a condition in which the weight vector is randomised in the tridimensional space and never has a constant direction for seconds or longer.

Empirical results obtained using simulated microgravity are frequently, though not always, found to be similar to those observed in true microgravity of space flight experiments. Most of the time the result of simulated microgravity are enhanced by true microgravity. This similarity is also present in different non-biological uses of the technique of time averaging of the weight vector as a simulator of microgravity. For example, the industrial production of microscopic spherical particles utilises this technique (Monodisperse Latex Reactor developed by NASA, [Roberts Glyn O., et al, 1991]). This similarity in the results, however, does not support a logical conclusion that all underlying mechanism were identically affected in both environments. It is estimated that the level of microgravity obtained with axial rotation to be around $10^{-2}\text{g}$ against the $10^{-4}$, $10^{-6}$ experienced in today's spacecraft. Theoretical and experimental projects are currently under way to improve cell culture techniques utilising simulated microgravity conditions. The focus of many of these efforts is to understand if a rotating vessel provides a sufficient "simulated microgravity" environment, or if a complex 3D rotational system is required. To literally experience "Zero g", that is to not experience any gravitational pull, an object would need to be infinitely far from any gravitating body. All other conditions of "weightlessness" result from a net sum of all forces present equalling zero, not from an absence of gravity. In the case of the clinostat or the rotating vessel, while it slowly rotates, the particles are strongly influenced by viscous drag and tend to rotate with fluid medium. Their motion relative to the rotating fluid is determined by a balance of their gravitational and centrifugal forces with forces exerted by the fluid, and the sign of the buoyancy forces depends on the density difference between the particles and the fluid. Moreover, the stress forces exerted by the fluid on the particle have to be separated into pressure force and drag force. Identifying an appropriate frame of reference is an important concept for understanding altered inertial environments, whether created by freefall in space or through rotation on Earth. Perfect weightlessness is difficult to achieve within a spacecraft therefore, “near weightlessness” on the order of $10^{-2}$ to $10^{-6}$ g is more typically experienced in today’s spacecraft and is usually referred to as microgravity (with “micro” defined either literally as $10^{-6}$, or figuratively as “very small”). Thus, one can consider the second constraint in studying the effects of gravity on a spacecraft to be the determination of whether or not this less-than-perfect weightless environment results in a sufficiently small force acting on the experimental system so as to be deemed statistically negligible.

**Microgravity Biology**

As already mentioned, the similarity in the results obtained in microgravity and simulated microgravity, does not support a logical conclusion that all underlying mechanism were identically affected in both environments. Biological effects attributed to gravity must ultimately be established as a cascade of events beginning with the altered inertial environment (physics), which potentially leads to altered structural loads (engineering) and/or electrochemical gradients (chemistry) being
generated, and concludes with the observed response of the organism (biology).

Research conducted in biological sciences supports human space exploration by using the environment of space as a unique laboratory for study of biological processes. Fluid dynamics is an area of major interest in biological science and great interest is devoted to its study in microgravity.

Fluid flows and transport of mass and heat occur in almost every biological process, from molecular and subcellular scale to whole system. Human exposure to long-duration low-gravity environments is known to produce many undesirable physiological effects. Change in vascular fluid distribution result quickly from the loss of hydrostatic pressure, and, on a longer time scale, from the shift of intercellular flows. Reduction of weight-bearing stress in microgravity induces bone loss and remodelling behaviour. An adequate understanding of the underlying fluid physics and transport phenomena can provide new insight needed to develop effective countermeasures.

Presently, we know that microweight does evoke a number of effects on cells, and a number of well-controlled experiments have shown that the absence of weight can have profound effects on fundamental biological processes active within the cell. In trying to understand possible mechanisms that contribute to these effects, it was helpful not to think of cell in transition from normal weight to microweight conditions, but to think of it in transition from microweight to a normal weight condition. How can it perceive weight? Weight would somehow cause a situation inside the cell of differential mass displacement, due to differences in the mass of cell-internal organelles and supramolecular structures. Such a condition of displacement should be of a sufficient magnitude to trigger the adoption by the cell of a new internal equilibrium. This very condition could be called the 'weight signal', the energy level of which will have to be above the local thermal noise inside the cell. The concept of weight signal, and its relation to mass displacements inside the cell, is put here in very general terms on purpose, because the detailed sequence of effects that causes the signal is not really important. What is important though, is that a minimal energy level is required to evoke any reaction in the cell. This translates into a minimal displacement of mass inside the cell and therefore to the notion that the weight vector has to have a constant direction for some minimal duration. The important conclusion from these considerations is: the notion that cells require a minimal time of unidirectionality of the weight vector to sense weight at all. The extracellular matrix (ECM) of mammalian cells, as mentioned, provides a complex environment. In this environment seems for some unknown mechanisms that the cells behave as they do not feel weight. The physical basis for these effects of microgravity on cells is thought to be due to the low-shear fluid environment that the cells and tissues encounter in real or simulated microgravity. By eliminating sedimentation, buoyancy and density driven convection, the cells can be grown in a relatively quiescent fluid environment, where interactions between cells can occur with minimal disruption. This would allow cell aggregates and tissues to form as in vivo but also suggests that methods are required, by which the mechanical environment may be modified to control the development of engineered cell structures. Extensive research on the effect of mechanical stimuli on cell metabolism suggests that tissues may respond to mechanical stimulation via loading-induced flow of the interstitial fluids. In a bioreactor, the cells are subject to a flow of culture medium. Flow properties such as flow field, flow regime (e.g. turbulent or laminar), flow pattern (e.g. circular), entity and distribution of the shear stress acting on the cells greatly influence fundamental aspects of cell function, such as regulation and gene expression. This has been demonstrated for endothelial cells and significant research efforts are underway to elucidate these mechanisms in various other biological systems. The endothelial cell (EC) layer, which lines blood vessel provide the principal barrier to the transport of water and solutes between blood and underlining tissue. ECs are continuously exposed to the mechanical shearing force (shear stress) and normal force (pressure) imposed by flowing blood on their surface, and they adapted to this mechanical environment. In low gravity, the mechanical environment of ECs is perturbed drastically, and the transport properties of EC layers are altered in response. It is proposed that alterations in mechanical forces induced by microgravity, and their resultant influence on transendothelial transport of water and solute, are largely responsible for the characteristic cephalad fluid shift observed in humans experiencing low gravity. Understanding the mechanisms behind this fluid shift is crucial to developing countermeasures for crews facing long-duration exposure to low-gravity. Another examples of flow and shear stress affecting mechanical and functional properties cells can be found in cartilage tissue.
Fluid Dynamics in Biology

Most of the biological, environmental, and industrial processes required to support life take place in a fluid phase. Fluid motion accounts for most transport and mixing in natural and industrial processes as well as in living organism. The ultimate goal of research in this area is to predict and even control this fluid behaviour. A detailed understanding of fluid dynamics over a range of length and time scale is essential for progress in many emerging research areas of physical and biological sciences. The low -gravity environment of space offers a unique opportunity for the study of fluid physics and transport phenomena, as the nearly weightless conditions allow researchers to observe and control fluid phenomena in ways that are not possible on Earth. Research on the behaviour of fluids and transport phenomena in weightlessness is also essential to the design and development of self-sustaining closed-loop system required for human life support.

In this light, and considering that biological effects attributed to gravity must ultimately be established as a cascade of events beginning with the altered inertial environment, the aim to investigate fluid dynamics is for understanding its role in the balance of the forces in the inertial reference environment of microgravity and simulated microgravity. A first goal would be to design a bioreactor that is capable to regulate several parameter of the flowfield inside the vessel. This capability would be of fundamental importance not only for better regulation of the force in the inertial environment in the vessel, but will also lead to knowledge on how cells are affected by different fluid flow. The possibility to investigate flow and shear effects on cells, provoked by controlled variation of flowfield and pressure, will help our understanding of differentiation, proliferation and organisation of cells.

Several studies demonstrate the sensitivity of cells to hydrodynamic stresses. Factor affecting the effects of fluid flow on animal cells are flow field (flow regime and flow patterns), level of 'shear', time of exposure, medium composition (eventual additives). One of the purposes to study the shear sensitivity of cultured animal cells is to have information that will allow one to define an appropriate environment in which the cells can be cultured at large scale. This involves the identification of the relevant parameters of the flow field governing hydrodynamic related to cellular behaviour, e.g. injury, and their relationship with the equipment geometry (bioreactor design) and operation. In an ideal situation cell injury should be predicted by knowing the actual stress that the cell experiences and, from intrinsic cell mechanical properties, the resulting cell deformation. Should the cell deformation exceed a critical value, disruption of the cell structure would be expected.

Flows to study the cell response to hydrodynamic stresses have been characterised by different parameters. Shear stress and rate of share were used to characterise the flow in well-defined flow devices (parallel plate chambers, concentric cylinders, and plate-and-cone viscometers). Average wall shear stress and specific power dissipation, and Kolmogorov eddy length scale have been used for agitated small-scale bioreactors. Operation parameters such as gas flow rate, bubble frequency, and bubble size have also been used to characterise sparged bioreactors. Even though these parameters have proved to be useful to correlate cell damages under the conditions of the particular experiment, their applications to reactor design questionable. For example, we do not know how to use parameter correlate cell damage in one particular system to predict cell damage in a different system. The problem reduces to asking what are the relevant parameters which govern hydrodynamic cell injury for any given flow. To date they have not been identified.

Parameter used to characterise hydrodynamic related cell injury should be of a general nature. These parameters cannot come from the geometry of the system producing the flow but from "intrinsic" characteristics of the flow itself. Any consideration of the geometry of the system producing the flow will limit their generality. Once intrinsic characteristics of the flow have been identified, their relationship with the geometry and operation of a given system can then be considered for optimisation purposes. A second requirement is that we should consider the local and not 'average' parameters. Cell injury in a hydrodynamic environment (assuming no nutrient and no mass transfer limitations) must result from the application of external stresses. Consequently, it must be a function of the local intensity of these stresses. Average values prevent the identification of those regions in the flow where cells can be killed. A flow may injure a cell at one point and have no effect at a different location. Thus, the values of the selected parameters must be local if general applications must be accomplished.

Here the attention is focused on cell injury as a measure of the effect of fluid flow on the cells. The reason of this choice is because of the amount of data on this effect coming from the great
importance of it in cell culture. Flow parameter of a general nature that can be used to study hydrodynamic related cell injury are suggested. The first step is to propose how an individual cell or a cell attached to a surface of a microcarrier can be damaged as a result of fluid flow. Cell damage for suspended cells is defined as the disruption of the cell structure (possibly the rupture of the cell membrane) that results from cell deformation. For anchorage-dependent cells, cell damage is defined as the detachment of cells from the surface support. This definition is motivated by the fact that anchorage-dependent cells cannot divide when suspended. In microcarrier culture was observed that once cells detach from their surface support, they lie in less than 2 hours.

Some experiment evidence that cultured cells may behave as viscous liquid droplets, however it does not imply that these cells have the same dynamics as true liquid droplets in an imposed flow. Because the cell interior is not a continuum, the measured viscosity is an apparent viscosity and the cortical tension is not a true interfacial tension. Nevertheless, considering a general deformable particle having a cortical tension and viscous interior, it is easy to see that a pure extensional flow will be more effective than a rotational one in deforming the particle. Flow characteristics associated with this "effectiveness" to produce deformation should be considered in our analysis of hydrodynamic related cell injury.

The forces to which a suspended cell or a cell on the surface of a bead may be subjected have been analysed in detail. Analysis of the fluid mechanics occurring suggests that there are actually three potential damage mechanisms: collision of a cell with other cells (or cell-covered microcarrier with other beads in case of microcarrier culture), collision with parts of the bioreactor (primarily the impeller in case there is one), and interaction with turbulent eddies the size of the cells (or microcarrier). Review of the available quantitative information on agitation effects in cell culture does not establish which mechanism is predominant. The negative effects of excessive agitation on tissue cells culture have often been ascribed to "shear".

In a turbulent liquid exist eddies of a range of sizes. This range of sizes is rather sharply bounded at one extreme by a smallest eddy size below which the kinetic energy of the eddies is rapidly viscously dissipated. The size and the velocity of these smallest eddies in isotropic turbulence are dependent by the kinematic viscosity of the medium and by the rate of turbulent energy dissipation per liquid mass. At steady state, the energy dissipated by turbulence equals that supplied by the agitator to the fluid, so the volume-averaged rate of turbulent energy can be calculated known the volume of the bioreactor.

A turbulent eddy much larger than the microcarrier beads can surround and rapidly accelerate a single bead to eddy's velocity or move groups of beads without creating a large relative velocity between them. With typical conditions the smallest eddy size is approximately 200 µm, or about the same as the beads size. In that case a bead would be too large for the smallest eddy to readily accelerate it, and the eddy would dissipate its kinetic energy against the cells on the bead's surface. Several eddies could also interact simultaneously with a single bead. Eddies approximately the size of this spacing would also be capable of increasing the number of bead-bead collisions by accelerating a bead into its neighbours. It can be shown that bead-bead collision are capable of delivering a physiological significant amount of energy to a cell. The effect of bead-bead collisions may be characterised by the turbulent collision severity (TCS), defined as the product of collision kinetic energy and frequency. Collision of the beads against internal part of the bioreactor have similar effects as bead-bead collision. In case of internal moving part the kinetic energy of the collision is much higher, because of the higher collision velocity.

Through an increase in viscosity, the turbulence can be damped and hydrodynamic effects can be reduced. Thickening agents which are completely soluble in cell culture medium and could significantly increase the viscosity in a flow with little or no toxic effect on cells, exist. However, for mammalian cells of interest, mechanical shear stress levels in the range 3 to 10 dyne/cm² cause damage to cells and reduce cell viability (Cherry RS, 1986).

### Simulated Microgravity Bioreactors

In static flat culture flasks or dishes the 2-dimensional environment cells tend to sediment in bidimensional layer and tend to alter gene expression and prevent differentiation. As was already mentioned through the report various techniques can be used to manipulate, counteract or partially alter the net effect that gravity has on an object (e.g. agitation, freefall, centrifugation, diamagnetic levitation, time averaging). All this techniques represent important tools in biotechnology to allow the researcher to isolate specific individual components of the collective effects induced by gravity. Against the effect of the
gravity on cells the technique of time averaging of
the weight vector is considered here the most
interesting and therefore some example of it are
hereafter shown.

Clinostat and Rotating Wall Vessel

The fundamental difference between
clinorotation and true freefall can be summed up as
follows: both environments can effectively (or
nearly) eliminate cumulative sedimentation for the
given object of homogenous mass suspended in a
sufficiently experiences chronic stimulation that is
time averaged to zero, while an actual freefall
environment essentially eliminates the stimulation
(and can therefore be said to result in an
instantaneous, rather than time averaged, net zero
force). Particle motion within a clinostat reaches
terminal velocity in which the accelerated motion
induced by gravity is balanced by the viscous drag
of the fluid medium. This is not the same as
weightless freefall, in which an object travels
unimpeded under constant acceleration (either
centrifugal or linear). In addition, coexisting objects
of varying density will not be equally balanced
within rotating system as they are in a state of true
freefall. If the objects of concern are of small
enough dimension and of a single density, they can
for all practical purposes, theoretically be
identically suspended in a sufficiently viscous
liquid medium in either environment.

Clinostat and Rotating Wall Vessel (RWV)
bioreactor are functionally similar devices in so
much as both operate on the premise that constant
rotation of the system normal to the gravitational
field of Earth will randomise the otherwise
unidirectional pull of gravity, and both devices
offer logical ground-based analogies for
augmenting space flight studies. The clinostatic
principle involved is that a fluid rotating (at the
appropriate rate) about a horizontal or nearly
horizontal axis (with respect to gravity) allows cells
or cell attachment substrates having a density
different from the fluid to travel in a nearly circular
path and to deviate insignificantly from the fluid
path. From the rotating reference frame the gravity
vector is observed to rotate so that its time average
is nearly zero. This allows for suspension of the
particles in a carrier medium with low fluid shear
and with low interference. The vessel wall is
rotated in order to reduce the adverse fluid velocity
gradient through the boundary layer at this wall
(which would occur at the interface between the
moving fluid and fixed wall). The rotating wall is
sufficient to cause fluid rotation due to viscosity.
The operating limits are defined by the
sedimentation rate of the particles in the fluid
medium and the acceptable centrifugal force due to
rotation. The vessel rotating horizontally around its
axis accomplish to a solid-body rotation of the
fluid. In a rotating bioreactor, the cells can be
fooled into thinking they are in a body. The RWV
bioreactor consists of a cylindrical growth chamber
with gas exchange membrane. The culture chamber
with diffusion gas exchange is completely filled
with culture medium (zero headspace). As the
vessel rotates, the liquid inside accelerates until the
entire fluid mass is rotating at the same angular rate
as the wall. Thus, this environment eliminates most
of the disruptive shear forces associated with a
conventional bioreactor. Microcarrier beads and
cells obey simple kinematics and are uniformly
suspended in the culture medium. The suspended
cells rotate as a solid body with minimal disruptive
shear forces (about 0.2 dyn/cm²), and the cells
maintain their relative position for long periods,
allowing them to touch one another or to construct
bridges between the microcarrier beads. In addition,
chamber rotation subjects the cells to a constantly
changing angular gravity vector. Constant
randomisation of the normal gravity vector subjects
to a microgravity environment and akin to the free
fall experienced for much shorter periods by
aircraft in parabolic flight. In this environment,
cells aggregate and undergo three-dimensional
growth to form tissue-like spheroids. As aggregates
grow during culture, the speed of vessel rotation is
increased to counter gravitational sedimentation.
This environment fosters tissue growth in three
dimensions. This bioreactor enables the growth of
clumps of cells that are larger in diameter.

The fluid dynamic operating principles of the
RWV culture system thus encompass solid body
rotation about a horizontal axis with some degree of
dimensional spatial freedom, oxygenation
without turbulence, high mass transfer rate, low
fluid shear forces, and the co-localisation of
particles that have different sedimentation rates.
The design requirement for the vessel to be used in
space are similar to those of ground-based
bioreactors, with a few exceptions. For the
mammalian cells of interest, mechanical shear
stress levels in the range of 3 to 10 dyne/cm² cause
damage to cells and reduce cell viability. At shear
level as low as 0.92 dyne/cm² cell proliferation,
morphology, and function are adversely affected.
Thus, to encourage three-dimensional growth and
differentiation, and to further study the effect of
shear on cells, shear levels of 10⁻² dyne/cm² are
desired. To meet this requirement, the flow field
must be laminar. In the microgravity environment,
the shapes of gas/liquid interfaces are dictated by surface tension. Gas bubbles form rather than the horizontal surface commonly used for gas exchange in bioreactors operated in Earth's gravity. This along with the concern of large stresses during dynamic coalescence of bubbles, lead to zero-headspace (no gas phase) requirement for the bioreactor. RWV in microgravity has also been shown to have limitations: It is difficult to remove air bubbles from the vessel on orbit without degrading the low-share culture environment or damaging the delicate three-dimensional tissue assemblies. Is limited in its ability to control the location of cells and tissue aggregates within the vessel. The RWV bioreactor, developed by NASA, was originally designed to protect cell culture from the high shear forces generated during the launch and the landing of the space shuttle.

Random Positioning Machine

What the Clinostat and Rotating Wall Vessel achieve in two-dimensional space, the Random Positioning Machine (RPM) produced by Dutch Space (former Fokker Space) achieves in three-dimensional space: the weight vector experienced by the organisms of an experiment continuously changes its direction in three-dimensional space with a frequency of more than 1 Hz. An experiment is mounted at the centre of a cardanic framework, the rotations of which are driven by two independent motors. The movement of the experiment is controlled by software providing four basic modes of operation: a centrifuge mode around one vertical axis only, two clinostat modes around one horizontal axis or around the other horizontal axis, perpendicular to the first one, and finally a random positioning mode, following a path of calculated positions based on numbers from a random generator.

In all cases, the so-called walk speed, defined as the speed of a given point on the experiment projected on a sphere of fixed diameter, can be set at a constant value. By adding selection criteria of the random positions generated, the machine can be instructed to avoid a succession of positions in the same space segment, which might distort the time-averaged microweight condition. Similarly, the machine can be instructed to spend more accumulated time in a particular space segment, resulting in average weight vectors of some pre-established values. Such a capability allows the investigator to impose onto the experiment values in between normal weight and microweight for the purpose of establishing threshold levels of the organism under study.

Conclusion

At the state of the art, the presence of a more controlled mechanical environment may be the condition required in order to study the biochemical and mechanical response of these biological systems. Such a controlled environment could lead to an advanced fluid dynamic design of the culture chamber that could both enhance the local mass transfer phenomena and match the needs of specific macroscopic mechanical effects in tissue development.

Moreover, a bioreactor that is used both for ground and flight experiments would provide the additional benefit of isolating the dependent variable of gravity. This continuity will provide a mean to compare results to a control experiment.

The bioreactor is an excellent example of how the skills and resources of two distinctly different fields can complement each other.

References
