SYNTHECON Whitepaper

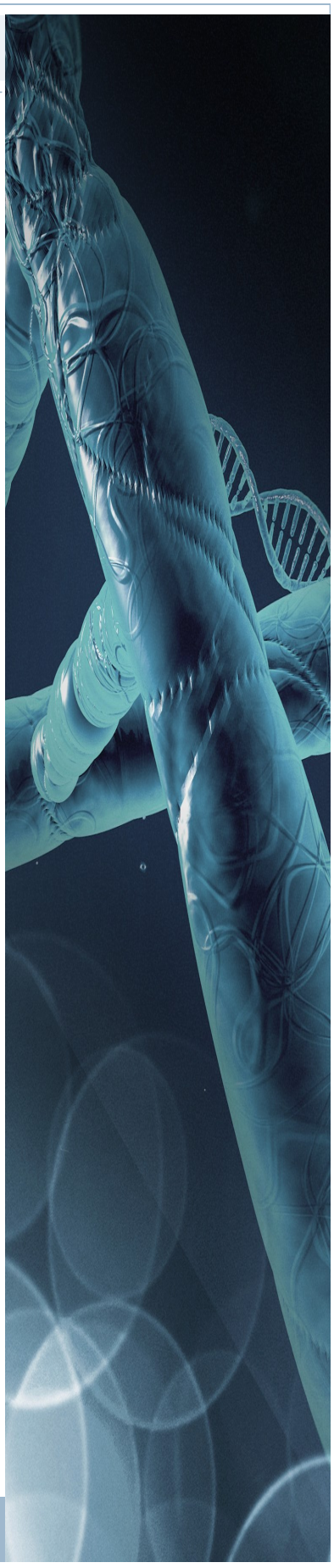
LOW SHEAR MICRO-GRAVITY– **A BETTER WAY TO DO 3D CELL CULTURE**

The *in vitro* culture of cells has played a central role in the biological sciences dating back to the beginning of the 20th century. Yet, for all the advanced tools that have been developed for studying cells, the method for culturing cells has remained virtually unchanged (1). The classical method for culturing cells involves growing cells as two-dimensional monolayers on flat, impermeable supports made of plastic or glass. While this method contributed much to our understanding of basic cell biology, it falls short of recapitulating *in vivo* structure and function of differentiated tissues. The key component that is missing in monolayer culture is growth in 3D. It has long been appreciated that the culture of cells in 2D monolayers leads to a rapid loss of the differentiated phenotype, but only in the last 10-15 years have methods to culture cells in 3D come into more widespread use.

3D CULTURE METHODS

A variety of technologies for cell and tissue culture have been developed since the first use of glass or plastic substrates. Some of these culture techniques have been adapted with varying degrees of success to 3-D culture. The simplest of these is a cell pellet in which isolated cells are packed in the bottom of a test tube by centrifugation and covered with culture media (2).

While this technique can produce 3-D cultures, diffusion of nutrients, oxygen and waste is limited by the static nature of the culture environment. Consequently, this technique has not been widely used. Another simple technique is to culture anchorage-dependent cells in a plastic flask or dish which has not been treated to promote cell attachment. As the cells float in the media, they may randomly aggregate and form large irregular clumps. Many individual cells, however, fail to make contact, become apoptotic and die. Furthermore, cells within the large aggregates will eventually become necrotic due to limitation of diffusion of nutrients and oxygen in the static culture environment. More recently, a number of investigators have used 3-D hydrogels to encapsulate cells. This arrangement has been shown to produce reasonable 3D tissue models by allowing cells to organize within the matrix (3). The limitations of this procedure are that it restricts mass transfer due to the static nature of the culture conditions and the matrix itself presents an additional barrier to diffusion. It is also not readily adaptable to scale-up. Another of the more recent methods is the hanging drop which resembles the floating cell aggregation technique except the cells are forced to aggregate in a small drop which creates a more controlled environment for producing uniform spheroids. The hanging drop culture has the same diffusion limitations as the other static culture techniques as well as being difficult to maintain in long term culture by changing media.



SYNTHECON

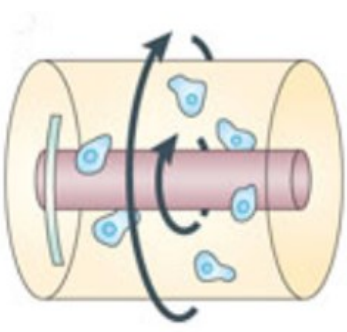
The Rotary Cell Culture System (RCCS) is a bioreactor technology that produces 3D cultures in a fundamentally different way than the aforementioned methods. First, it is a dynamic system which suspends cells in a low-shear stress, microgravity-like environment (see Principles of Operation of the RCCS below) allowing anchorage-dependent cells to readily aggregate into 3D spheroids while simultaneously producing high mass transport of nutrients and oxygen (4). Unlike spinner flasks, the RCCS suspends cells without cell damaging mechanical force (5). The RCCS can also be used with a variety of scaffolds.

The RCCS was originally developed at NASA, Johnson Space Center to simulate the microgravity conditions in space. It was based on the principle of clinorotation, defined as the nullification of the force of gravity by slow rotation around one or two axes. The clinostat developed at NASA is a single axis device known as the Rotating Wall Vessel (RWV). The RCCS is the commercial version of this device.

" The RCCS is a dynamic system that suspends cells in a low-shear stress, microgravity-like environment."

PRINCIPLES OF OPERATION OF THE RCCS

The RCCS is a horizontally rotated cylinder with a coaxial oxygenator positioned in the center (Fig. 1). The vessel is completely filled with culture media and when rotated, the fluid flow is coupled to the vessel wall such that it rotates essentially as a solid body. The oxygenator core is fixed and rotates at the same angular velocity as the outer wall and thereby creates a laminar flow with minimal shear force. Cells placed in this environment are maintained in suspension by the resolution of the gravitational, centrifugal and Coriolis forces (Fig.2). As the vessel rotates, the cells or cell aggregates accelerate until they reach terminal (sedimentation) velocity at which the gravitational force is counterbalanced by hydrodynamic forces of shear, centrifugal force and Coriolis force. The major determinant of sedimentation velocity is the size of the cell aggregate which, according to the Stokes equation increases as the square of the radius (4). Therefore, as cell aggregates grow in size, they will sediment more rapidly and it is necessary to increase the rotational speed of the RCCS to maintain aggregates in suspension and avoid damage from wall collisions.



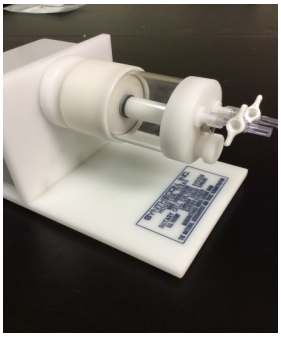


Figure 1. Horizontal rotation of the cylindrical vessel creates a microgravity-like environment (left). An image of an RCCS vessel attached to the motorized rotator base (right). The vessel and rotator base operate inside a standard humidified CO_2 incubator.

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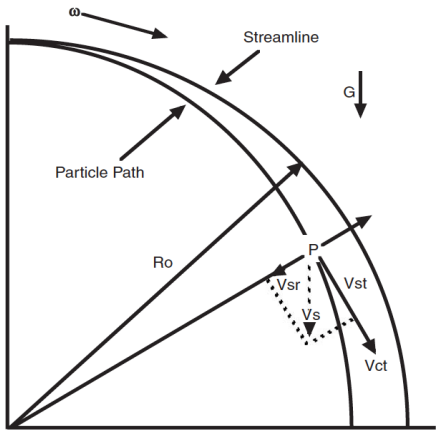

Figure 2.

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Figure 2. The forces acting on a particle (P) rotating in a fluid are shown. Gravity-induced sedimentation (Vs) can be resolved into radial (Vsr) and tangential components (Vst and Coriolis, Vct). There is an outwardly directed vector due to centrifugal force.

HOW THE RCCS STACKS UP AGAINST COMPETITION

Encapsulated Cells

Neural stem cells encapsulated in 3D collagen gels in static multiwell plates (6) were able to differentiate into neurons and form functional synapses (7). Nevertheless, after a certain period, the cells became necrotic due to inadequate diffusion of nutrients and oxygen in static culture. When the encapsulated cells were placed in the dynamic culture environment of the RCCS (Fig. 3) they not only survived for much longer periods (Fig. 4) but formed complex brain-like tissues (8,9) (Fig. 5).

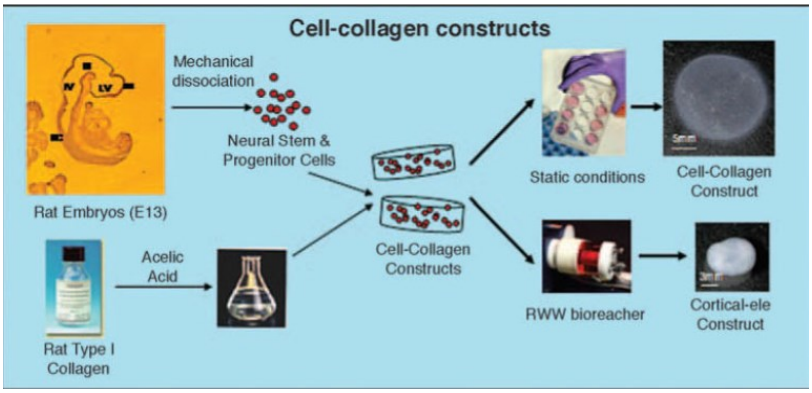


Figure 3.

Figure 3. Neural stem and progenitor cells were isolated from rat embryonic day 13 cerebral cortex, encapsulated in collagen and cultured in static multiwell plates or RCCS as described in ref. 9.

SYNTHECON

Figure 4. Initially neural cells in static culture proliferated more rapidly, but eventually died off due to the limited diffusion of nutrient and oxygen. In contrast, the RCCS-cultured neural cells continued to proliferate and maintained viability for a prolonged period in culture. (ref 9)

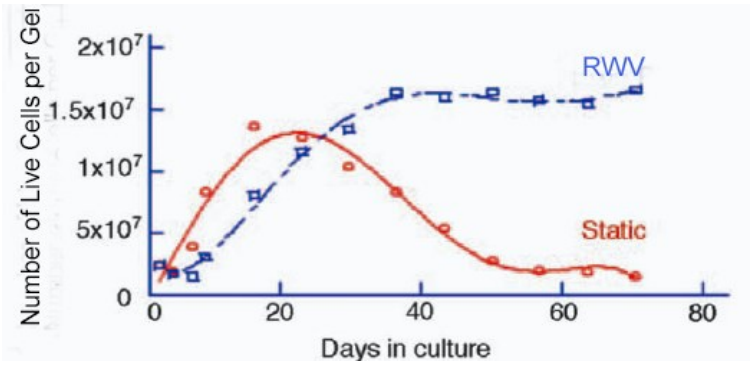


Figure 4.

Figure 5. In the developing rat cerebral cortex (a), the proliferating Nestin+PCNA+ neural progenitor cells form the neuroepithelium (NE) while the differentiating neuronal and glial cells are found in the cortical plate (CP) region. In the RCCS cell-collagen constructs (b), layer I contained nestin+ and PCNA+ cells corresponding to the NE while layer II contained TuJ1+ neuronal cells similar to the CP region. (ref. 9)

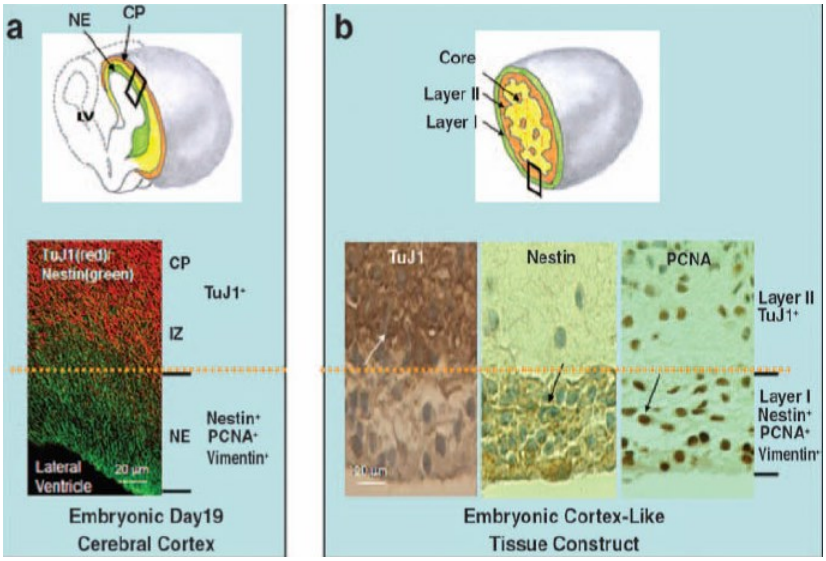
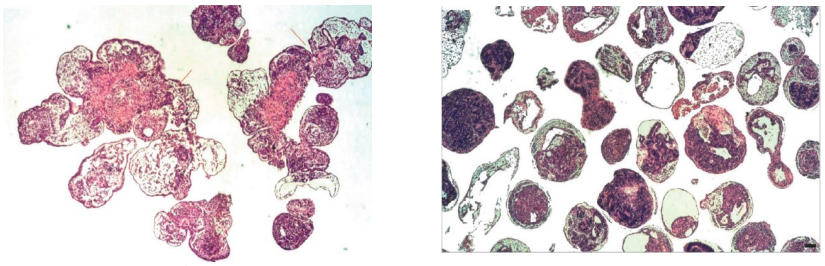


Figure 5.

Cell Suspension in Static Non-adherent Plates

The ability to form embryoid bodies composed of cells from all three germ layers is considered a fundamental characteristic of embryonic stem cells. One early method of generating embryoid bodies was to allow the cells to form aggregates in non-adherent dishes (10). This method, however, was difficult to control and tended to form large, irregular aggregates with necrotic centers due to the limitation of diffusion in static culture. When embryoid bodies are produced in the RCCS they form smaller, more uniform spheroids with improved cell survival and enhanced cell differentiation (11, Fig. 6).

Figure 6. Comparison of embryoid bodies produced in static and RCCS culture (ref.11)



Static RCCS

Figure 6.

Hanging Drop Culture

Hanging drop culture suspends cells in a drop of media hanging by surface tension to a solid support. Cells within the small volume of the drop tend to aggregate and form 3D spheroids. As a static culture method, the hanging drop has the same diffusion limitation as other types of 3D static culture. When hanging drop culture was compared to RCCS culture in a study of differentiation of embryonic stem cells to cardiomyocytes, culture in the RCCS produced more efficient differentiation as demonstrated by the increased expression of cardiac troponin T (12), Fig. 7.

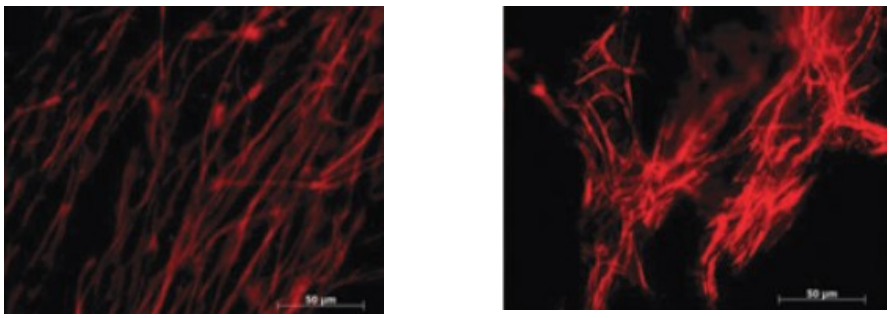


Figure 7.

Figure 7. Embryonic stem cell spheroids produced in hanging drop or RCCS cultures were placed in differentiating media for 5 days and immunostained for cardiac troponin T.

For more examples of the advantages of dynamic 3D culture in the RCCS, see the Research Publications section of Synthecon's web site, www.synthecon.com.

"When compared to hanging drop culture, the RCCS produced more efficient differentiation and expression of the gene of interest."

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