The Rotary Cell Culture System™
RCCS-1/RCCS-4

The Premier Tissue Engineering Culture System

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User's Guide

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Introduction

The Rotary Cell Culture SystemTM (RCCS™) is new technology for growing either anchorage dependent or suspension cells in the laboratory. The system enables the researcher to cultivate many types of cells to high densities. Cells that could not be grown easily by other methods have been grown without difficulty in RCCS™ vessels. The growth of co-cultures of differentiated 3D aggregates that mimic the structure and function of the parent tissues can be achieved in the RCCS™. The system provides a reproducible complex 3D *in vitro* culture system for the investigation of the structural processes and regulatory molecules that control differentiation in normal tissues and transformation in neoplastic tissues. Some articles in scientific journals have used the term"organoids" to describe the 3-dimensional differentiated tissue models and expanded surgical explants that have been grown in the RCCS™. Developed at the Johnson Space Center, the system was originally designed to protect delicate tissue cultures during spaceflight. However, it quickly became apparent that the unique environment of low shear force, high mass transfer, and simulated microgravity, provided by the system enables 3D cell growth to take place in a conventional laboratory tissue culture incubator. Two basic types of RCCS™ vessels are manufactured by SYNTHECON, Inc. The Slow Turning Lateral Vessel (STLV™) is tubular shaped and has a central gas transfer core and is designed for use primarily with anchorage dependent cells. The High Aspect Ratio Vessel (HARV™) has a disc shaped culture chamber with the oxygenator membrane forming the inside wall of the vessel. The HARV™ was designed for use with suspension cells. However experience has shown it to be an excellent culture vessel for anchorage dependent cells and tissue explants as well.

Principles of Operation

Tissue Culture in the Rotating Cell Culture Vessel

The Rotary Cell Culture System is a horizontally rotated, bubble free culture vessel with membrane diffusion gas exchange. The culture medium, cells and cell aggregate particles rotate with the vessel and do not collide with the vessel walls or any other damaging objects when rotated at appropriate speed. Destructive shear forces are minimized because this system has no impellers, airlifts, bubbles, or agitators. Suspension cells, anchorage dependent cells on scaffolds or primary tissue explants establish a uniform, very low shear, fluid suspension orbit within the horizontally rotating culture vessels. As the cells proliferate, the rotation speed is adjusted to compensate for increased sedimentation rates. The

absence of damaging stress forces allows three dimension aggregation of large cell masses. Normal, neoplastic, mono and co-cultures of fragile, human, anchorage dependent and suspension cells have been grown *in vitro*. One of the primary advantages of the new Rotary Cell Culture System over conventional systems is its ability to grow tissue cultures that differentiate or mimic the structure and function of the parent tissue. The investigator can inoculate the culture with the same mixture of cells, which normally occurs *in vivo*. These cells grow and organize into three dimensional tissue aggregates similar to the parent tissue. Such growth is possible because destructive turbulence and collision forces are minimal within the horizontal Rotary Cell Culture System. Surgical explants or biopsies can be transferred into the RCCS™ and be maintained without necrosis.

This extremely low shear culture environment can be used to grow virtually any cell type that can be cultured in conventional culture systems (see the bibliography at www.synthecon.com for examples). The SYNTHECON, Inc. Rotary Cell Culture System (RCCS™) enables a scientist with a standard tissue culture laboratory to produce very complex, differentiated, tissue-like constructs. As a bonus, users who need to continuously monitor their cell cultures can easily take samples at very short intervals without damaging the culture. The effects of changes to a cell culture caused by the addition of other cell types, chemotherapeutic agents, nutrients, chemicals, or consumer test products can be easily observed. Harvesting the culture is simply a matter of removing the end cap and pouring out the culture or aspirating it with a pipette. No release agents or scrapers are required.

Notes and Cautions

Please read before using

Please complete and return the Limited Warranty Sheet on page immediately. The Warranty will not be valid unless it is signed and returned to Synthecon.

Do not soak any part of the vessel in bleach, acidic or basic cleaning solutions. The plastic and rubber in the vessels can be impregnated with toxic chemicals from such solutions. rendering them toxic to cells. As an example, chlorine bleach will permanently poison the vessel.

Corrosive chemicals such as chromates will damage metal parts. Abrasive cleaners or strong organic solvents such as acetone will destroy the plastic and void the warranty.

The oxygenation membrane is particularly susceptible to damage. The oxygenator membrane is a very delicate component composed of silicone rubber, 0.005 inches thick covering the polyester cloth backing. When inserting pipets or any type of instrument into the vessel while culturing cells, avoid touching the membrane. Even the smallest breach of the membrane will render the vessel unusable and require that the vessel be returned to Synthecon for repair. During cleaning, use latex gloves and gently clean the membrane with the fingertip. With proper handling the membrane should last for many culture cycles before requiring replacement.

Storage of the Rotator Base in an incubator when not in use will result in damage to the motor and will void the warranty.

The ribbon power cable passes easily between the incubator door and gasket without compromising the incubator environment. The power supply can be conveniently located on top of or beside the incubator. ***The power supply must never be operated inside of the incubator.***

Subjecting the vessel to a strong vacuum by forcefully pulling back on syringes attached to the ports could burst the oxygenator membrane and require replacement of the membrane.
Do not over tighten bolts and fittings. Excessive force will strip the threads on the plastic components.

An autoclave cycle of 121^{O} C for 30 minutes is recommended. Exceeding this temperature will accelerate degredation of plastic components.

Residues in dirty autoclaves may inpregnate the oxygenator membrane and plastic parts and cause the vessel to become toxic.

The vessel should be autoclaved as an assembled unit. This reduces the risk of contamination during

assembly.

The central core assembly screw of the Slow Turning Lateral Vessel (STLV™) should be backed off one turn before autoclaving.

The peripheral screws on the High Aspect Ratio Vessel (HARV™) should be released one turn before autoclaving.

After autoclaving, be sure to tighten the screws which have been loosened.

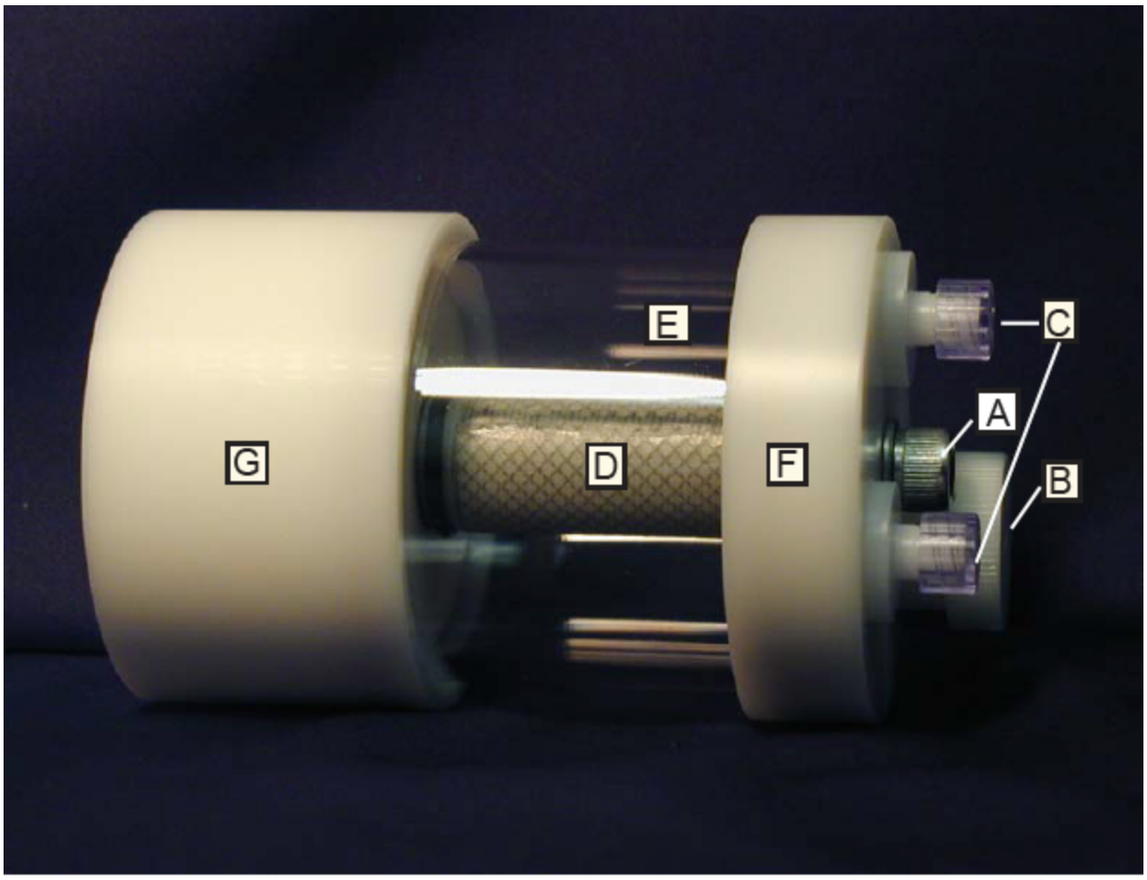
Since media formulations vary with each application, no recommendations are made as to which medias will work best with a particular cell type. In general, medias which work well in other culture systems will work well in the RCCS.

The humidification of the incubator in which the RCCS is placed must be maintained. Even though the RCCS is a closed culture system, evaportation of fluid can occur through the oxygenator membrane. This is particularly important with the 10 ml HARV™.

Bubbles cause turbulence which can damage cells. Any small bubbles which form during the culture should be removed as soon as they are observed.

Cells will readily attach to microcarriers or other scaffolds while the vessel is rotating. It is not necessary to pre-attach cells to scaffolds in static culture.

Slow Turning Lateral Vessel (STLV)



A. Center Bolt
B. 1/2" Fill Port Plug
C. Syringe Ports
D. Oxygenator Membrane and Core
E. Vessel Wall
F. Front End Cap
G. Rear End Cap

Microcarrier Preparation

If using microcarrier beads with anchorage-dependent cells, prepare the microcarriers in accordance with the manufacturer's instructions. The cells and microcarriers can be mixed and pipetted into the vessels together. Cells will readily attach to the microcarriers while the vessel is rotating. It is not necessary to stop the vessel rotation during the attachment phase. Attachment of cells to the microcarriers is normally complete within 24 hours.

Vessel Preparation

Note: Do not soak any part of the vessel in bleach, acidic or basic cleaning solution. Vessels will absorb such solution, rendering them toxic to cells. Abrasive cleaners or strong organic solvents such as acetone will destroy the plastic, voiding the warranty.

1. Using the Allen wrench supplied, unscrew and remove the center bolt (A) on the front end cap (F). Remove the end cap and the vessel wall. Unscrew the bolt on the rear end cap and separate the oxygenator core (D) from the rear end cap taking care not to damage the membrane on the core. Remove o-rings from both end caps.
2. Place all vessel parts in a large beaker or other container filled with laboratory detergent. Allow the parts to soak for at least 1 hour.
3. Scrub plastic parts (except oxygenator core) with a soft brush to remove any residues.
4. Gently clean the oxygenator core with the tip of the finger using latex laboratory gloves.
Note: Harsh scrubbing will damage membrane material. Do not use a brush to clean the membrane.
5. Thoroughly rinse vessel parts with tap water.
6. Soak vessel parts in ultra pure water (Milli-Q or equivalent) overnight.
7. Air dry the vessel parts.
8. Assemble vessel.
9. Sterilize as described below.

Sterilization

Autoclave Method

1. Remove fill port cap and autoclave separately from vessel.
2. Place plastic caps or aluminum foil on syringe ports to protect from contamination during reassembly.
3. Loosen center bolt 1 turn with Allen wrench tool supplied.
4. Place vessel and cap in autoclave bag or wrap with foil and autoclave for 20 minutes at 121°C.
5. Remove from autoclave and allow to cool.

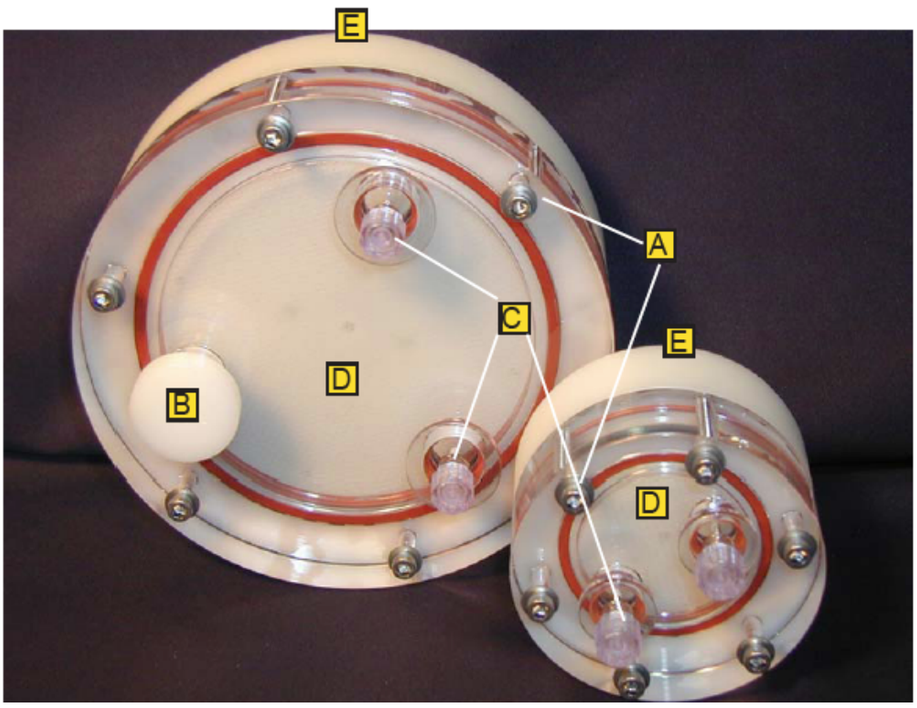
Experimental Start-up

1. Transfer the vessel to a laminar flow hood. Place the fill port plug on a sterile alcohol swab.
2. Attach sterile valves to the syringe ports.
3. Fill the vessel partially with media leaving enough space to add cells and scaffolds or microcarriers.
4. Add scaffolds or microcarriers via the fill port.
5. Add cells. If desired, cells and microcarriers can be mixed and added together.
6. Fill the remaining volume of the vessel with media and insert the fill port plug. There will always be small bubble remaining after this step. It is necessary to remove this bubble before starting the culture in order to minimize turbulence.
7. Bubble removal. Fill a 3 or 5 ml sterile syringe with media and attach to one of the valves on a syringe port. Attach a 3 or 5 ml sterile syringe on the valve of the other port. Open both valves. Maneuver the bubble under the empty syringe port and inject media from the filled syringe. Repeat this procedure until all bubbles are removed from the vessel. Close both valves and replace caps.
8. Attach the vessel to the rotator base.
9. Place the rotator base with vessel in a humidified CO_2 incubator. Attach the power cable to the back of the rotator base and to the power supply.
10. Turn the power on and adjust the rotational speed. If using scaffolds which are visible to the naked eye, the speed should be adjusted so that the scaffolds are maintained in suspension without touching the vessel walls. If suspended cells or microcarriers are being used, the inital speed can be set at approximately 10 rpm. Anchorage dependent cells will form aggregates and the rotational speed may have to be increased to compensate for the increased sedimentation rate as the aggregates increase in size.

Medium Replacement

1. Turn off the power, remove the vessel from its base and take it to a laminar flow hood.
2. Stand the vessel vertically with the valves facing up and allow the scaffolds,beads or cell aggregates to settle to the bottom.
3. Remove the fill port plug and place it on an alcohol swab.
4. Aspirate approximately 3/4 of the media being careful not to remove any cells.
5. Fill the vessel with fresh media and remove bubbles as described above and return the vessel to the rotator base in the incubator. Resume the rotation.

High Aspect Ratio Vessels



A. Peripheral Screws

B. 1/2 " Fill Port (55 ml only)

C. Syringe Ports

D. Front Plate

E. Back Plate

With a few exceptions noted below, the procedures for the High Aspect Ratio Vessel are the same as with the Slow Turning Lateral Vessel.

1. The smaller vessels do not have a fill port. Consequently, filling of these vessels must be done through the syringe ports. This also limits the size of the scaffolds to those that can be introduced through the syringe ports.

2. The High Aspect Ratio Vessel is held together with small screws around the periphery of the Front plate. Each of these screws should be loosened during autoclaving and then tightened before adding media. *Do not overtighten these screws.*

2. When changing media, the vessel should be placed vertically in the hood with the fill port at the top and secured to prevent it from rolling. The cell aggregates or scaffolds are allowed to settle at the opposite end of the vessel from the fill port. The vessel should then be propped at a shallow angle to keep the aggregates or scaffolds at one end and the fill port opened. A pasteur pipet connected to a vacuum source is then introduced through the open fill port and as much media as possible removed without disturbing the concentrated aggregates or scaffolds at the other end of the vessel. Fresh media is then added, the fill port plug replaced and the bubbles removed as described with the Slow Turing Lateral Vessel.

Equipment Usage Rights

SYNTHECON™ Inc. grants the purchaser a non-exclusive right to use the Rotary Cell Culture System equipment solely for the purpose of conducting research and specifically excluding use of this equipment for any purpose other than research. Synthecon technology is not intended for use on/in humans. Any desire by end user to manufacture commercial products in Synthecon, Inc. technology will require the end user to obtain a *User's License* from the National Aeronautics and Space Administration and/or Synthecon, Inc. Its use must comply with all laws, ordinances, and regulations relating to the possession, use, or maintenance of the equipment, including registration and/or licensing requirements, if any.

Patents in Force

The Rotary Cell Culture System™ is protected by patents exclusively licensed from the National Aeronautics and Space Administration (NASA) and patents owned by Synthecon Inc., with others pending. The patents Synthecon Incorporated operates under are listed below:

• Patent number 5,437,998 "GAS PERMEABLE BIOREACTOR AND METHOD OF USE" Patent issued August 1, 1995
• Patent number 5,665,594 "GAS PERMEABLE BIOREACTOR AND METHOD OF USE" Patent issued September 9, 1997
• Patent number 5,702,941 "GAS PERMEABLE BIOREACTOR AND METHOD OF USE" Patent issued December 30, 1997
• Patent number 5,763,279 "GAS PERMEABLE BIOREACTOR AND METHOD OF USE" Patent issued June 9, 1998
• Patent number 4,988,623 "ROTATING BIO-REACTOR CELL CULTURE APPARATUS" Patent issued January 29, 1991
• Patent number 5,026,650 "HORIZONTALLY ROTATED CELL CULTURE SYSTEM WITH A COAXIAL TUBULAR OXYGENATOR" Patent issued June 25, 1991
• Patent number 5,153,131 "HIGH ASPECT RATIO VESSEL AND METHOD OF USE" Patent issued October 6, 1992
• Patent number 5,155,035 "METHOD FOR CULTURING MAMMALIAN CELLS IN A PERFUSED BIOREACTOR" Patent issued October 13, 1992
• Patent number 5,153,133 "METHOD FOR CULTURING MAMMALIAN CELLS IN A HORIZONTALLY ROTATED BIOREACTOR" Patent issued October 6, 1992
• Patent number 5,998,202 "MULTIPLE CHAMBER DIFFUSION VESSEL" Patent issued December 7, 1999
• Patent number 5,989,913 "CULTURE VESSEL FOR GROWING OR CULTURING CELLS, CELLULAR AGGREGATES, TISSUES AND ORGANOIDS AND METHODS FOR USING THE SAME" Patent issued November 23, 1999

Alterations
Alteration of the equipment voids the warranty on this equipment. In no case shall SYNTHECONTM, Inc. be responsible for any modifications or alterations to this equipment performed by anyone other than SYNTHECON™, Inc.

IMPORTANT NOTICE

Limited Warranty: Limited Liability

SYNTHECONT™ Inc. warrants that, for one year, under normal operating conditions and use, this equipment will be free from defects of materials and workmanship. SYNTHECONTM Inc. will repair or replace defective parts at our option. Contact SYNTHECONTM Inc. immediately upon discovery of a defect. SYNTHECON™ will provide you with a return authorization number and shipping instructions.

Components

Oxygenator Membrane
The oxygenator membrane is a very delicate component consisting of silicone rubber, .005 inches thick, covering a polyester cloth backing. Care and attention should be given to the membrane during cleaning, sterilization, and removal of cultured material. Synthecon reserves the right to make discretionary determination as to the cause of damage with returned oxygenators, and deem whether the repair is covered under the Synthecon Limited Warranty. See Operators Manual for appropriate procedures.

Rotator Base
Storage of the Rotator Base in an incubator while not in use will result in damage to the rotator components. Synthecon reserves the right to make a discretionary determination as to the cause of damage with returned rotators, and deem whether the repair is covered under the Synthecon Limited Warranty.
The equipment must be used and operated in a careful and proper manner. In no event shall SYNTHECON™ Inc. or its suppliers be liable for any indirect, special, or consequential damages, including but not limited to, loss of cells, medium, data, labor or equipment incurred by the purchaser or any third party arising from the use of, or inability to use this equipment.

Service
For service during and after the expiration of the warranty, contact SYNTHECONTM, Inc. at (713) 741-2582 during 9 a.m. to 5 p.m., US Central Time Zone. Equipment being returned for service should be shipped to: Synthecon,- Customer Service Dept, 8044 El Rio, Houston, TX. 77054. Please include a short description of the problem, service required or reason for the return. Please pack equipment being returned in sturdy containers with adequate packing materials. Synthecon will not be liable for damage sustained during shipment.

SYNTHECON™, Inc. also provides biology and engineering contract support services. Special custom designed equipment can be built to meet the customer's needs. Customers can provide cell samples of their cell and tissue lines, and SYNTHECON™ Inc. will conduct growth and feasibility studies of the customer's cells on a contract fee basis. Sub-licenses are available which would include design, scaleup, and manufacture of production equipment.

Copying and Sale

Duplication, modification or sale of copies of this equipment is prohibited. This equipment is patented by the U. S. Government. SYNTHECON™ Inc. holds the exclusive licenses to these patents.

Acceptance of Equipment

The purchaser shall inspect the equipment delivered and immediately notify the seller of any discrepancies with the equipment. If the purchaser fails to provide notice in writing within 14 days after the delivery of the equipment, the purchaser will be presumed to have accepted the equipment. The acceptance and use of this equipment constitutes an agreement upon the purchaser's part to the usable condition of the equipment.

Refurbished Products

Refurbished products carry a separate warranty, this warranty does not apply. For details of refurbished product warranty, please refer to the refurbished product warranty information packaged with each refurbished product.

WARRANTY WILL NOT BE VALID IF IT IS NOT SIGNED AND RETURNED.

Warranty valid to original purchasers only. Please sign and return by mail immediately to: **For international locations see the very last page**

Synthecon, Inc.
Customer Service Department
8044 El Rio
Houston, TX 77054

Purchaser: _____

Institution/Organization: _____

Purchase Date: _____

Invoice/PO#: _____/_____

Model #: _____

Serial Number(s) _____

(PLEASE NOTE THAT THIS PAGE IS FOR YOUR RECORDS.
PLEASE USE THE YELLOW PAGE THAT ACCOMPANIES THIS MAUAL FOR WARRANTY REGISTRATION!)

14 International Locations

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