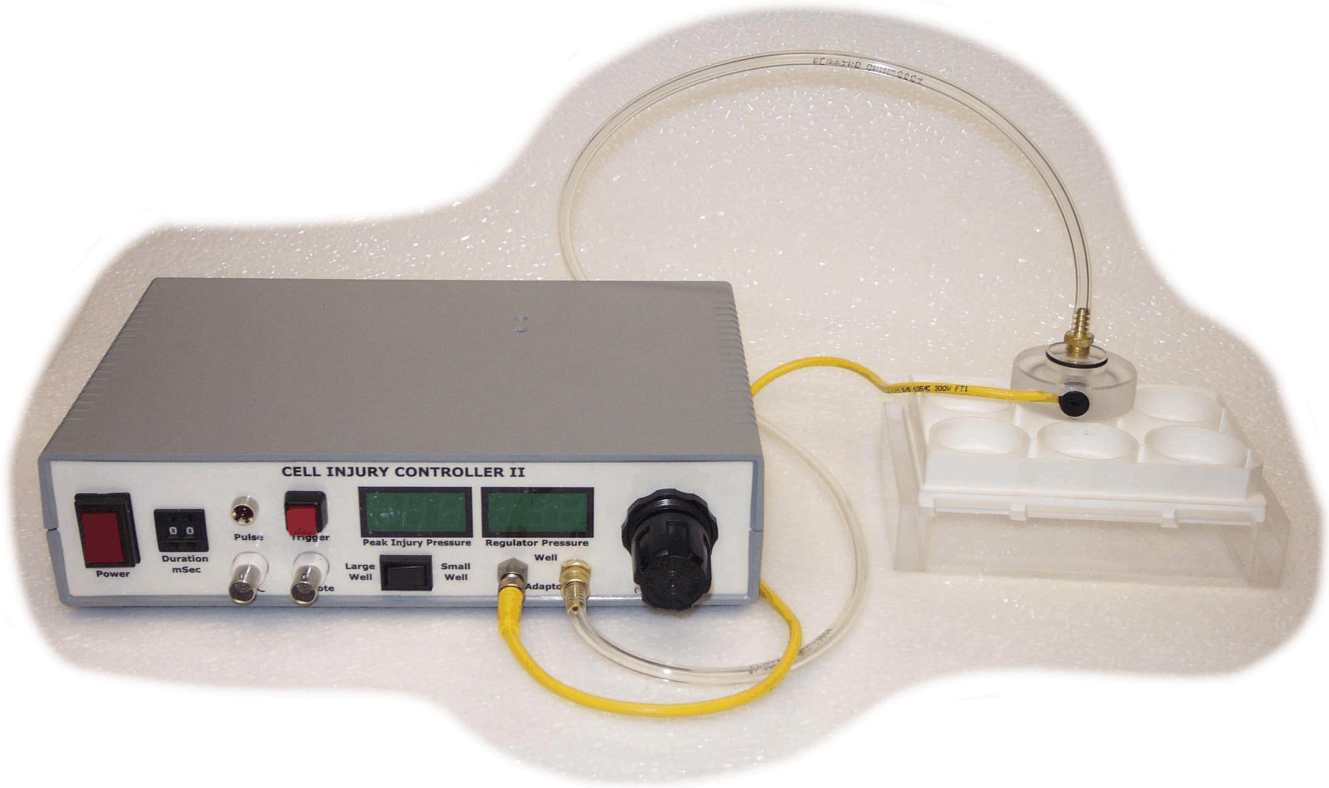
Custom Design & Fabrication Incorporated

Giving structure to **your** ideas

Cell Injury Controller II
(CIC II)



CIC II Operation Manual

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Introduction

The Cell Injury Controller II is an electronically controlled pneumatic device for producing strain induced trauma to *in vitro* cell cultures. Tissue cultures are grown in commercially available trays and controlled gas pressure is used to deform the elastic bottom of the tray resulting in quantifiable biaxial stretch of the cells adhered to the elastic surface.

Quick-Start

This manual contains detailed information on the controls and functions of the Cell Injury Controller II but it can be very dry reading. Fortunately, the device is quite simple to operate and the Quick-Start section should get you up and running in a matter of minutes. The Quick-Start provides a basic description of the device connection and operation. You can refer to the detailed descriptions if anything needs clarification or you don't find something intuitively obvious.

- Connect the pressure source and power to the CIC II.
  Power - 100-240 Volts AC or DC
  Gas Source - 60 to 80 PSI (414-552 kPa) not to exceed 100PSI (690 kPa).

- Attach the well adapter plug.
  The manual's cover is a good reference.

- Turn the power switch on.

- Place an **empty** culture tray in the plate holder.

- Select the appropriate well size.

- Set the delay (50mSec recommended).

- Set the regulator pressure to 15 PSI (a safe starting point).

- Insert the well plug and hold it firmly in the tray.

- Trigger the device a couple times and note the peak pressure.

- Adjust the regulator pressure and trigger the device until the desired peak pressure is obtained. (See the injury chart on page 10.)

- Place the subject culture in the clearance spacer and insert the adapter plug.

- Trigger the device and record the peak pressure.

Theory of Operation

Cells are cultured on an elastic membrane in special trays. The CIC II applies a controlled flow of gas to pressurize a selected well. The cell culture will experience a biaxial stretch injury as the pressure in the well deforms the membrane to which the cells adhere. The CIC II allows control of injury severity by regulating the gas flow rate and duration to effect a volumetric change in the selected well. The peak pressure developed in the well is captured and provides an indication of the exact stimulus applied.

Injury Measurement Method

The CIC II monitors the pressure developed in the culture well to measure the force applied to the elastic membrane. The biaxial stretch of the elastic membrane is a function of the applied force, the elasticity of the membrane. The CIC II provides control of biaxial stretch and the rate of stretch development to control the injury severity. The ***Flex I***® and ***BioFlex***® culturing trays from Flexcell International Corporation have proven to provide consistent results with this method.

The CIC Model 94A used the ***Flex I***® tray and measured linear displacement of the elastic membrane as the reference of the biaxial stretch. The elastic culture area of the ***Flex I***® tray was molded in the tray and provided consistent displacement. The ***BioFlex***® trays however, use a two piece clamp to hold a sheet of ***Silastic***® material which allows minor membrane slippage that makes linear displacement inconsistent at the pressures used to create cell injury. The change from linear displacement to pressure measurement overcomes this limitation and allows the CIC II to provide accurate results with either culturing tray type.

Monitoring the force applied to the well has several advantages over measuring motion of the membrane:

- Pressure monitoring produces accurate results with either tray type.
- Ease of use. There is no complicated setup and the measurement of injury is automatic. Changing tray types is a simple matter of reversing the well adapter plug and setting the well size selector switch.
- Pressure transducers are non-contact devices so measuring peak pressure does not affect the motion of the membrane. Measurements are fast and accurate.
- A consistent and automatic method is used to record the stimulus applied. Errors of measurement interpretation are eliminated.
- The stimulus applied is recorded for every injury. Mechanical or procedural differences are eliminated as sources of inconsistency. For example, varying fluid levels between wells, or movements of the well plug would result in differing gas volumes and therefore different injury pressures. This could be an elusive source of inconsistency if the applied pressures were simply assumed equal rather than actually measured.

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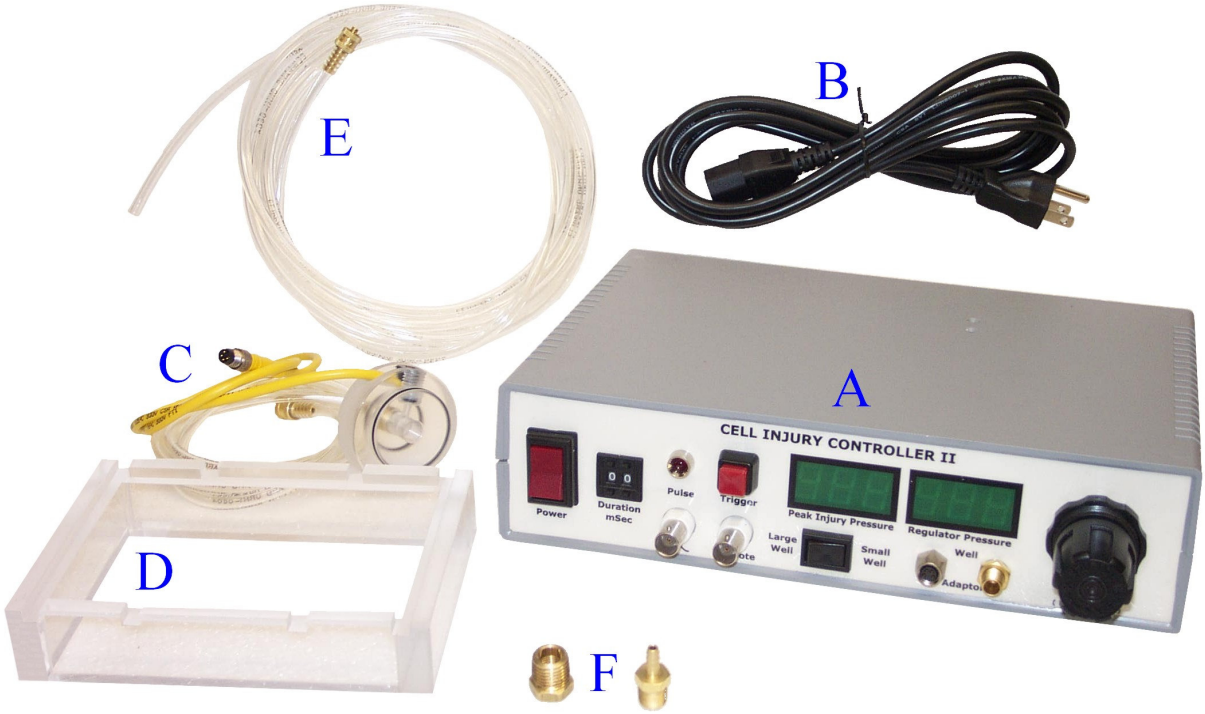
Components - The Cell Injury Controller II includes the components pictured below.

A. Cell Injury Controller II
B. U.S. 115VAC CSA Power Cord
C. Pressure Plug and Transducer
D. Culture Tray Holder
E. Gas Source Connection Tubing
F. Source Adaptor Fittings



External Requirements - The CIC II requires electrical power, a pressurized gas source, and elastic bottomed cell culturing trays.

Electrical Power - The CIC II accepts 80 to 240 Volts AC or DC. An appropriate CSA power cord is all that is required to connect to the supply. The standard U.S. power cord (B) is supplied.

Pressure Source - The source gas may be any non-corrosive gas appropriate for your application such as air or nitrogen. The recommended source pressure is 60 to 80 PSI (414-552 kPa) and should not exceed 100PSI (690 kPa). A 10 foot connection hose (E) and adapters (F) are provided for this connection. ***Gas cylinder pressures are in the thousands of pounds. A tank regulator is required.***

Culturing Trays - The CIC II accepts the following culturing trays from Flexcell International Corporation:

Flex I® 29.45cm^2 culturing tray (also used with the CIC Model 94A)
BioFlex® 57.75cm^2 culturing tray

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Front Panel

Power - Standard power control switch operation. On is up. Down is off.

Duration (mSec) - This sets the length of time that gas flows into the well. It is adjustable from 01-99 milliseconds. When the switch is set to 00 the device is in manual mode and the solenoid will follow the trigger. This is useful for applying a constant pressure for measuring the stretch associated with a given pressure. In manual mode, the device will inhibit the trigger and display HPE (High Pressure Error) if you attempt to trigger the device when the regulator pressure is beyond safe levels for continuous application at the selected well size.

Pulse - This is an indicator(top) that illuminates when the gas solenoid is active and an analog voltage output(below) from the pressure transducer in the well plug. Connection to an oscilloscope or other analog capture device will allow monitoring the rate at which pressure changes in the well.

Trigger & **Remote** - The pushbutton switch or a remote contact initiate the injury pulse. The remote input will accept either a switch contact closure (such as a remote pushbutton) or a TTL/CMOS compatible digital signal as the trigger input. The trigger is active low.

Peak Injury Pressure - This displays the peak pressure from the well plug. The peak will be held until the state of the device changes. Changing any of the switches resets the peak value. In manual mode, this displays the current plug pressure and peaks are not captured.

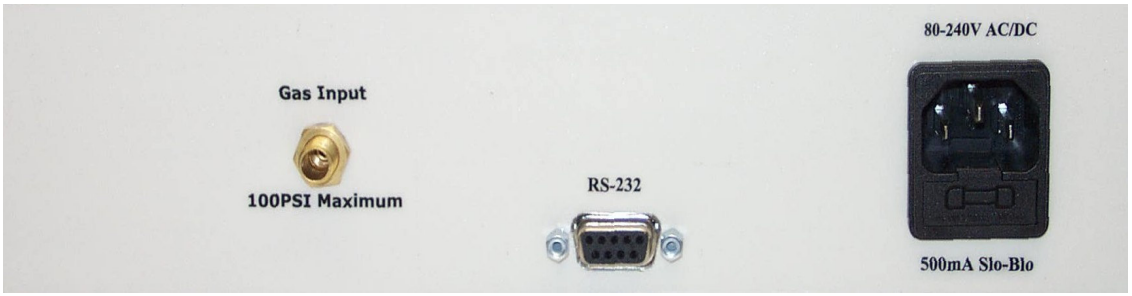
Regulator Pressure - This displays the gas pressure being supplied to the metering solenoid. The pressure setting determines the flow rate through the metering solenoid.

Large Well / Small Well - This determines the gas metering to be used for inflation and deflation. ***Ensure this switch is set correctly. The gas volume and pressure limits for the two tray types are vastly different.***

Well Adaptor - These are the connections to the well plug adaptor. The electrical connection to the pressure transducer is on the left and on the right is the connection to the gas metering solenoid.

Regulator - This adjusts the gas pressure to the metering solenoid. (Adjustment tip: Trigger the device periodically when adjusting the regulator to relieve the pressure and allow the regulator to stabilize.)

Rear Panel



Gas Input - This is the connection for the pressurized gas source. The recommended source pressure is 60 to 80 PSI (414-552 kPa) and should not exceed 100PSI (690 kPa). ***Gas cylinder pressures are in the thousands of pounds. A tank regulator is required.***

RS-232 - A serial port is provided to facilitate custom control or data logging. Contact Custom Design & Fabrication if you have need of this functionality.

Power Input - Connect an appropriate CSA power cord. The CIC II accepts 80 to 240 Volts AC or DC. The standard 115VAC U.S. power cord is supplied.

Connections

Connect the gas source - The typical gas source is a high pressure gas cylinder with a tank regulator. ***Gas cylinder pressures are in the thousands of pounds. A tank regulator is required.*** A 1/4 inch NPT to 1/8 inch NPT reducer, 1/8 inch hose to 1/8 inch NPT connector, and 10 foot of connection hose are supplied for this purpose. Use PTFE thread sealing tape for all bare metal to metal pipe thread connections. Connectors with rubber seals do not require sealing tape but a light coating of petroleum jelly on the hoses and rubber seals will make them easier to insert and increase their longevity. The hose will then be connected to the rear panel. (PTFE is the abbreviation for polytetrafluoroethylene. It is best known by the trade name of ***Teflon***®.)

Adjust the source gas pressure - The recommended source pressure is 60 to 80 PSI (414-552 kPa) and should not exceed 100PSI (690 kPa). If you are using a gas cylinder and regulator, turn the regulator adjustment counter-clockwise until you feel the spring tension stop. This will ensure that the regulator is off and will not exceed the maximum input pressure of the CIC II. Open the tank valve and then adjust the tank regulator for the correct pressure.

Configure the well plug - The well plug fits either the ***Flex I***® or the ***BioFlex***® trays by threading the connector into the unused side of the plug. The connector does not need thread sealing tape or excessive tightening. The connector will seal in the acrylic plug with just a snug connection.

Connect the well plug - The gas and transducer connections for the well plug adapter are below the display.

Select the well size - Set the well size switch to match the culture tray you plan to use.

Connect the power cord - Turn off the power switch and connect the power cord.

Operating Procedure

The steps to prepare the device and injure the subject cell culture are as follows:

- Connect the pressure source and power to the CIC II.
- Attach the well adapter plug.
- Turn the power switch on.
- Place an empty culture tray in the plate holder.
- Select the appropriate well size.
- Set the delay (50mSec recommended).
- Set the regulator pressure to 15 PSI (a safe starting point).
- Insert the well plug and hold it firmly in the tray.
- Trigger the device a couple times and note the peak pressure.
- Adjust the regulator pressure and trigger the device until the desired peak pressure is obtained. (See the injury chart on page 10.)
- Place the subject culture in the clearance spacer and insert the adapter plug.
- Trigger the device and record the peak pressure.

Operation and Setting Details

When the power switch is turned on, it will take a few seconds for power supply start up and device self test. As soon as the display comes on, the device is ready for use.

The severity of injury is controlled by regulating the gas flow rate and flow duration. The peak pressure developed in the well is captured and provides a measure of the exact stimulus applied.

Flow rate is a function of the pressure applied to the metering solenoid. The front panel regulator is used to set the pressure and thereby control the rate of inflation. Note that pressure regulators exhibit large hysteresis(they don't respond immediately). Triggering the device periodically while adjusting the regulator will relieve the pressure and allow the regulator to stabilize. The typical pressure settings range from 20 to 40 PSI.

Duration is the amount of time the solenoid valve is open and gas is allowed to flow into the culture well. We suggest a duration of 50 mSec simply because this setting has been utilized is a large portion of the published studies. (Maintaining a standard whenever possible is always helpful in comparing your results with others.) With a fixed duration, the regulator pressure is used to adjust the level of injury. The CIC II contains a combination of high speed solenoids and metering valves for inflation and deflation of each well size. These are adjusted to make the settings corresponding to a given injury as consistent as possible between the two types of culturing trays and between CIC II and the original CIC Model 94A.

Injuring the culture requires enough clearance for the elastic expansion of the culture well. The clearance spacer provided has recesses to hold either tray type and provides the required clearance for the membrane expansion below the culture well.

Insert the well plug into the desired well and double check the well size selector switch position. The pressures for each size are quite different and selecting the wrong size could cause the membrane to burst.

When you press the trigger switch, you will need to hold the adapter plug firmly in the well. The tapered sides of the culture well and the pressure developed require you to hold the adaptor plug firmly to prevent the pressure pulse from popping the plug out of the well.

The peak pressure developed will be captured and displayed. The captured value is the actual pressure from the culture well and will therefore be an accurate reference of biaxial strain (stretch) regardless of variations in setup or procedure.

Result Analysis

Peak injury pressure is the indicator of biaxial stretch. All the other values are simply the settings used to achieve the peak pressure for your particular configuration. The settings will vary between devices and setups whereas peak injury pressure translates directly to biaxial stretch. The correlation is not a linear function but every experiment that obtains the same peak injury pressure for the same tray type will have the same biaxial stretch applied to the cell culture. If you maintain a consistent pulse duration you are assured that the stimuli are as close to identical as possible.

The chart below correlates peak well pressure to empirical data on the resulting injury severity obtained with the CIC Model 94A. You will need to determine the exact peak pressure required for the desired injury in your particular protocol but this should provide a good starting point.

Flex I Injury Settings
(Small Well with 1 ml of fluid media)

Injury level	Approximate Membrane Stretch	Model 94A - Flex I membrane deformation (dry reference well)*	Approximate Regulator Pressure	CIC II - Flex I equivalent injury peak pressure
Low	120%	5.5 mm	22 PSI	8.6 PSI
Mild	135%	6.5 mm	28 PSI	10.2 PSI
Severe	155%	7.5 mm	34 PSI	11.8 PSI

BioFlex Injury Settings
(Large Well with 2 ml of fluid media)

Injury level	Approximate Membrane Stretch	Model 94A - Flex I membrane deformation (dry reference well)*	Approximate Regulator Pressure	CIC II - BioFlex equivalent injury peak pressure
Low	120%	5.5 mm	18 PSI	1.8 PSI
Mild	135%	6.5 mm	28 PSI	2.7 PSI
Severe	155%	7.5 mm	44 PSI	4.0 PSI

*Note that the CIC Model 94A Flex I deformation data is the measured distension of a dry reference well because this was the original method of calibration. The corresponding peak pressure values are based on the anticipated injury deformation with the addition of 1ml of fluid media in the Flex I well and 2 ml of fluid media in the BioFlex well. The quantity of fluid affects the gas volume of the well and therefore the regulator setting required for a specific injury. The peak injury pressure is the important value. It is the peak pressure in the well that indicates the resultant stretch. The regulator pressures listed are only a reference to reduce the trial and error in achieving the desired injury pressure.

Additional Information

Publications dealing with biaxial strain Cell Injury of brain derived cells in culture can be found at:

http://www.people.vcu.edu/~eellis/CICPubs.html

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