ShellPa

Standard Mechanical Cell Stretch System
Model No: NNMS
Serial #:

User Manual

To operate the system properly and safely, read the manual before using ShellPa.

This system is not a medical device. For RESEARCH USE ONLY

Manufactured by:

Menicon Life Science

B-Bridge International, Inc.
20813 Stevens Creek Blvd, Suite 200, Cupertino, CA 95014
www.b-bridge.com ▪ customersupport@b-bridge.com ▪ +1-408-252-6200

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Safety Precautions

In case of misuse of ShellPa, it may lead to a risk of severe injury and/or breakdown of the system.
Only use ShellPa with the an air compressor
Turn OFF power when not in use
Read the entire ShellPa manual before assembling and using the instrument
Read the operating manual for JUN-AIR air compressor as well.
Only use the designated air pressure. Using other values may lead to damaging the instrument.
Keep away from flammable agents
Do not touch with wet hands
Instrument and air compressor should not be operated in standing water

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1. Introduction

ShellPa was designed to replicate the dynamic cellular environment of the body by applying mechanical strain to cultured cells. Physiological cell response to mechanical stress is a more realistic model than conventional static cell culture.

2. ShellPa Components

Main Unit

Controller

Air compressor
(with BMF integrator/mist filter and 2nd air pressure regulator attached)

Intake air filter

BMF Integrator/Mist Filter and
2nd Air Pressure Regulator

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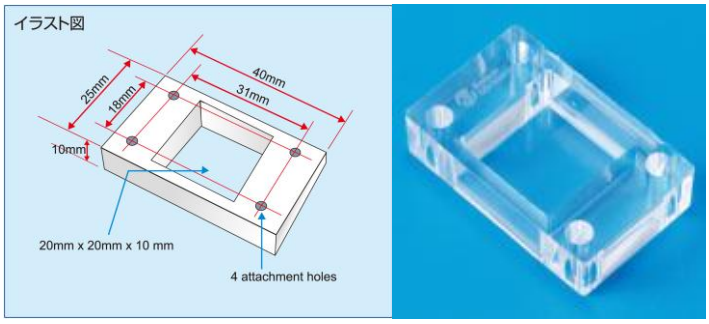
Red and Blue Air Tubes
Black Air Tube with air cock valve
White Air Tube (Exhaust tube) with noise suppressor
AC Adapter (Model: US301210), AC100-240V 50/60Hz 0.3AOP: DC12V

Transformer
For air compressor

Plastic Square Dish
For seeding cells in stretch chambers and covering chambers in Main Unit



Disposable Stretch Chamber, SC4B
Plasma treated

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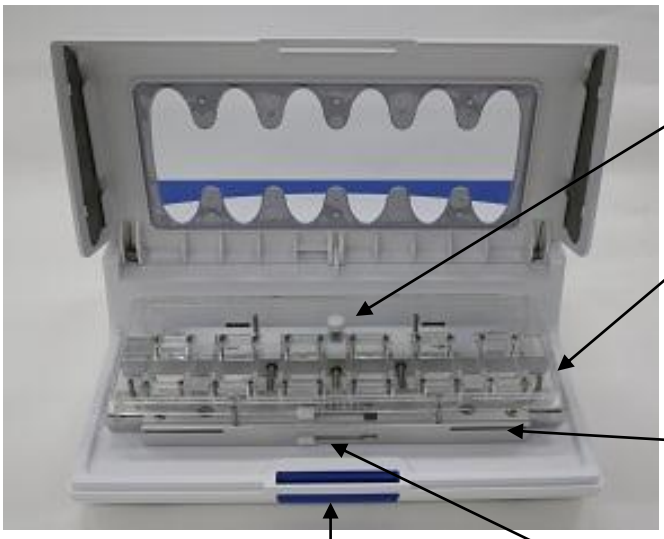
3. Component Descriptions

Main Unit



Offset Knob
For putting in or taking out Chamber Holder

Plastic Square Petri Dish to seed cells in the stretch chambers and cover chambers in Main Unit.

Chamber Holder
Holds 1 - 6 stretch chambers

Button to open cover

Stretch Ratio Adjuster

Controller

Read out of experimental conditions

Control Dial
Turn dial to set stretch frequency. Range is 1 cycle/min to 120 cycles/min. The default value is 60 cycles /min.

Start/Stop Switch

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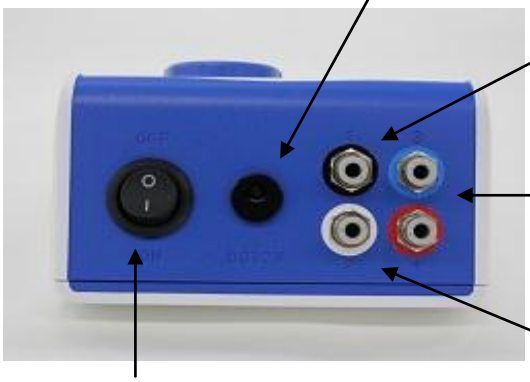
Back panel of Controller



Connecting Port for AC Adapter

Black Connecting Port
Black tubing with air cock valve connects to air compressor

Blue & Red Connecting Port
Blue & Red tubing connects to Main Unit

White Connecting Port
White tubing with noise suppressor

Power Button

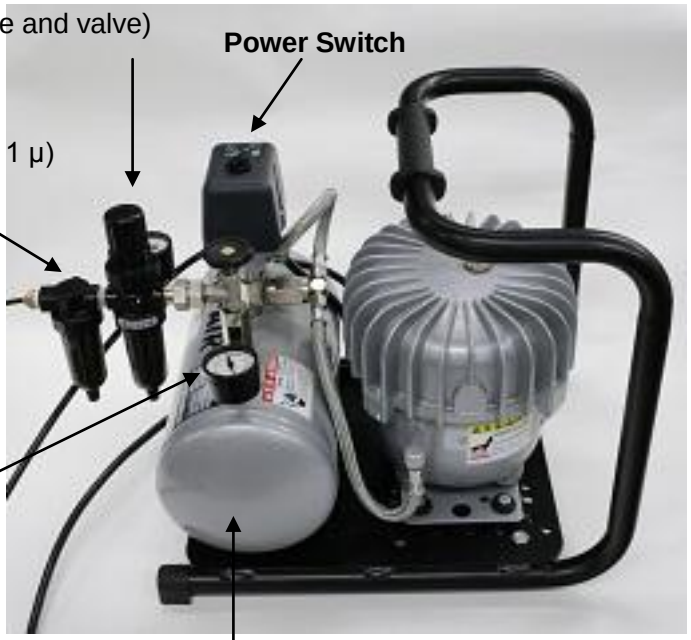
JUN-AIR 3-4 MC Air Compressor

2nd Air Pressure Regulator (gauge and valve)

Power Switch

BMF Integrator/Mist Filter (5 μ, 1 μ)

Connect to Controller via Black tubing with air cock valve



1st Air Pressure Regulator (gauge and valve)

Single Phase 100V 0.15Kw Tank 4L
Working Pressure: 0.5MPa
Switchback Pressure: 0.7MPa

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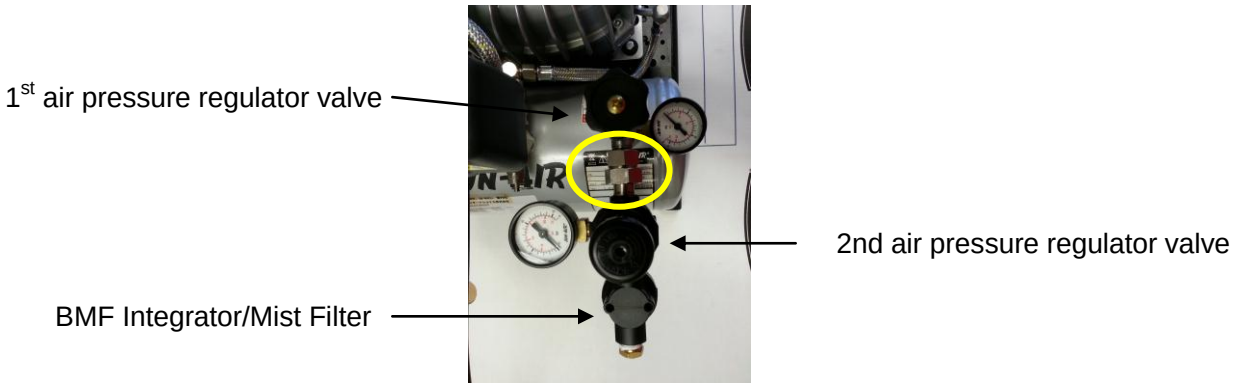
4. Set Up and Shut Down of Air Compressor and ShellPa

AT THE POINT OF PURCHASE, THE CHAMBER HOLDER IS LOADED ON THE MAIN UNIT. THE MAIN UNIT MUST BE PRESSURIZED TO UNLOAD THE CHAMBER HOLDER. READ THE ENTIRE USER MANUAL BEFORE USING SHELLPA OR ATTEMPTING TO ASSEMBLE OR DISASSEMBLE THE INSTRUMENT.

Set up air compressor:

1. Take off the yellow and black protective cap on the side of the air compressor and replace it with the intake air filter.

2. Screw the BMF Integrator/Mist Filter-2nd Air Pressure Regulator into the 1st Air Pressure Regulator. Tighten the adjoining screw.



3. Make sure all the valves are in the CLOSED position
    a. First air pressure regulator: turn CLOCKWISE to close
    b. Second air pressure regulator: pull up knob, turn COUNTER CLOCKWISE to close, push down knob to lock position
    c. Waste valve: turn CLOCKWISE to close

Waste value

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4. Make sure both drain tubes (thick yellow tube and black tubing) drain into a waste container. The small black tubing may already be connected to a plastic bottle.

Waste tubes

5. Check oil level in the Air Compressor. If oil is below the minimum (MIN) level, add SJ-27 oil through the oil inlet

Oil inlet

6. THE COMPRESSOR IS MADE FOR 110 VOLTS AND NEEDS A STEP DOWN TRANSFORMER IF USING A 220/240 VOLTS POWER SOURCE. PLUG THE AIR COMPRESSOR INTO AN ELECTRICAL TRANSFORMER. INCLUDED WITH THE TRANSFORMER IS A PLUG ADAPTER.

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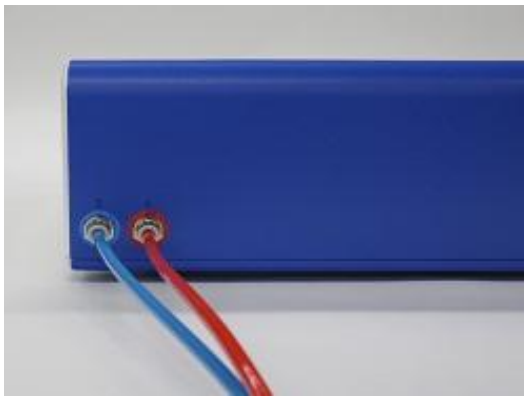
Set up Main Unit and Controller:

Depending on the ShellPa model, the location and orientation of the ports (blue, red, white, black) may be different than the image below. For all models, the color of the port corresponds to the color tubing it connects to.

Back panel of Controller

Back or side panel of Main Unit

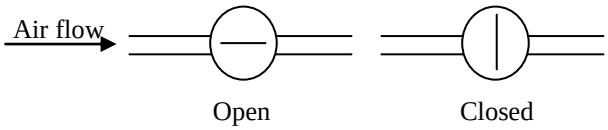


1. Use the Blue and Red tubing to connect the Main Unit to the Controller by gently pushing the tubing into the same color port. To release the tube from the port, push in the grey plastic washer surrounding the tubing, then pull out the tubing.

2. Connect the White tubing with the noise suppressor to the White port on the back panel of the Controller.

3. Connect the Black tubing with air cock value to the Controller. The air cock valve should be closer to the Controller and not the air compressor. The air cock valve should be in the **CLOSED** position (+). Attach the other end of the tubing to the BMJ Integrator/Mist Filter on the air compressor.

Air cock valve



Side view of BMJ Integrator/Mist Filter

Attach Black tubing to BMJ

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4. Plug the AC Adapter into the Controller. The AC Adapter is made for both 110 and 240 volts therefore no transformer is needed. Plug the transformer (with air compressor) and AC Adapter into a 240 volt power source.

Turn ON Air Compressor and ShellPa:

CAUTION: This system is pneumatic. The misuse of the system may lead to a risk of breakdown. Incomplete connections may cause an air leakage. Be sure to confirm the proper connections before operating the system.

1. Turn ON Air Compressor Power Switch. The green light next to intake air filter will illuminate.

2. OPEN the valve of 1st regulator by turning the valve COUNTER CLOCKWISE. The pressure will build to 7 Bar on the regulator. When the pressure reaches 7 Bar, the compressor will automatically turn off and the green light will also turn off.
    a. The regulator will indicate pressure regardless of the valve being open or closed. Therefore the measurement of pressure in the regulator is not necessarily an indication that the valve is open.

1st regulator at 7 Bar

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3. OPEN the 2nd regulator by lifting the knob up then turning it CLOCKWISE. Continue to turn the knob until the regulator reaches 3-4 Bars.

4. Once 2nd regulator reaches the desired pressure, lock the position by pushing down on the knob.

2nd regulator at 3 Bar

5. OPEN air cock valve (-) on black tubing leading to Controller. This will pressurize the ShellPa.

6. When the air pressure in the 1st regulator lowers to 5 Bar, the compressor will automatically turn on until it pressurizes to 7 Bar, then the compressor will turn off.

7. Turn ON the Controller using the toggle switch on the back panel.

8. Turn the knob on the Controller to adjust frequency. Press the START/STOP button to start or stop a run.
    a. The Controller must read “STOP” to change the frequency. Default setting is 60 cycles/minute.
    b. Turning the knob during a “Run” will change the display. The knob will toggle between current run and total run time.

9. Routine checking of air pressure is highly recommended during operation.

10. Guidelines

Valves	Open	Closed	Working Pressure
1st regulator	Counter clockwise	Clockwise	7 Bar
2nd regulator	Lift up, turn Clockwise, Push down	Lift up, turn Counter clockwise, Push down	3 – 4 Bar*
Air cock valve	Counter clockwise	Clockwise	-
Waste valve yellow tube	Counter clockwise	Clockwise	-

*2nd Regulator:
DO NOT pressurize over 5 Bar for safe use

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When operating the Main Unit for long periods of time at frequencies of 60 cycles/minute (1Hz) or faster, lower the pressure to 2 Bar to reduce load on the air compressor

Turn OFF Air Compressor and ShellPa:

1. Turn OFF the Controller using the toggle switch

2. CLOSE the air cock valve (+) on the black tubing.

3. Remove the BLACK tubing from the Controller by pushing in the grey plastic washer surrounding the tubing. This will release the tube.

4. CLOSE the 1st regulator by turning the valve CLOCKWISE

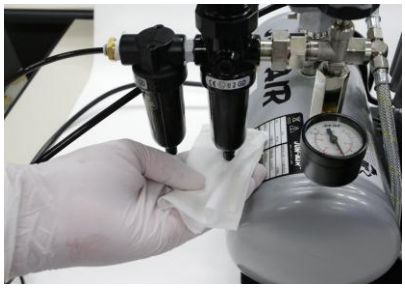
5. OPEN the air cock valve (-) on black tubing to release pressure. Decrease in pressure will be indicated by the 2nd regulator.

6. CLOSE the 2nd regulator by lifting up the knob, turn COUNTER CLOCKWISE until it stops turning, push down to lock position.

7. Release pressure from the air compressor tank by OPENING the waste valve. Turn the valve COUNTER CLOCKWISE. Pressure will decrease in 1st regulator. The air compressor motor may automatically turn on. Leave the motor ON until the fluid collected in the air pressure tank is flushed out the waste tubing.
    a. The air compressor compresses the atmospheric air and absorbs the moisture in the air that causes the fluid to pool in the tank. A periodical fluid removal from the tank drain and mist filters is required. In case of interfusion of such a fluid into the main unit or/and controller may lead to a risk of breakdown of the system.

8. Once pressure in 1st regulator reads zero and waste fluid is expelled, turn OFF motor

9. Blot the bottom of the BMJ filters with an adsorbent towel.




10. CLOSE the waste valve by turning CLOCKWISE

11. Unplug the Controller and air compressor from the power source

12. Unplug the tubes (red, blue, white, and black with air cock valve) from the Main Unit, Controller, and air compressor by pushing in the grey washer which will release its grip on the tubing.

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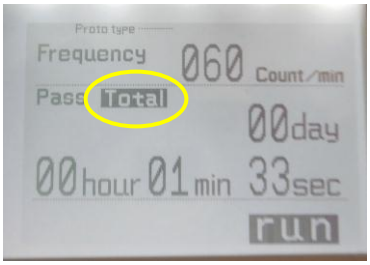
13. When transporting the air compressor or if not using for extended period of time, replace the intake air filter with the yellow protective cap to prevent oil leakage and dust interfusion.

5. Operation of ShellPa:

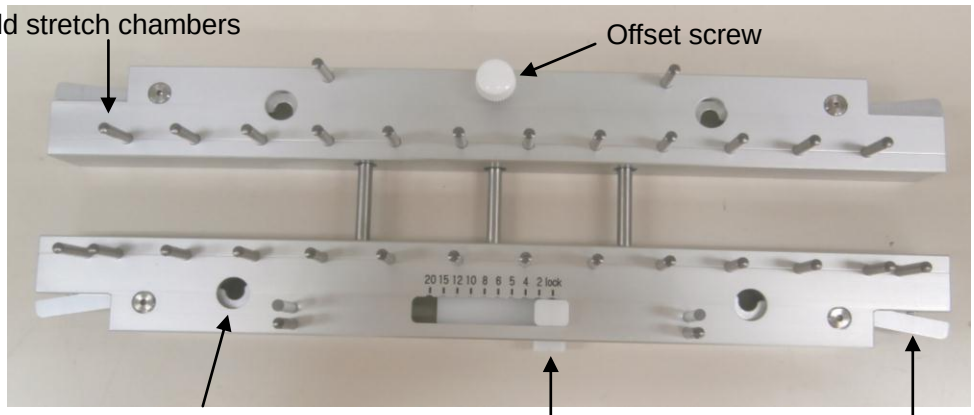
DISINFECT THE SHELLPA WITH 70% ETHANOL WIPES. ALL SURFACES THAT POTENTIALLY CAN HARBOR CONTAMINANTS SHOULD BE CLEANED PRIOR TO USE.

1. After the ShellPa is properly pressurized, turn the knob on the Controller to adjust frequency from 1 – 120 cycles/minute. The Controller must read “STOP” to change the frequency. The default setting is 60 cycles/minute.

2. Press the START/STOP button to start or stop a run. Turning the knob during a “Run” will change the display to show either time of current run or total time of run. The current time is reset every time the run is STOP. The total time will reset to zero when the power switch on the back panel of the Controller is turned OFF.



3. In the Main Unit, turn the Offset Screw CLOCKWISE to properly align chamber holder with support arms. Depress the Unload levers on each side of the holder, and then lift the chamber holder out of the Main Unit.
    a. Depending on the ShellPa model, some models need to be pressurized (connected to air compressor) to be able to unload/load the chamber holder from the Main Unit.



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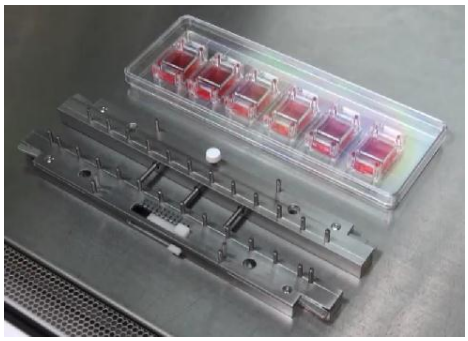
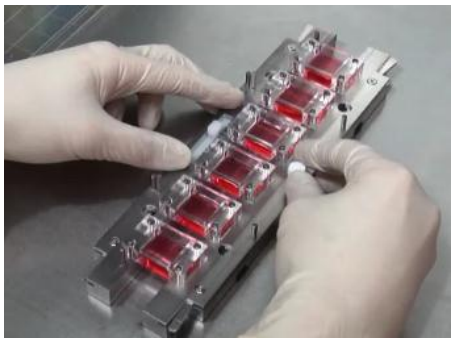
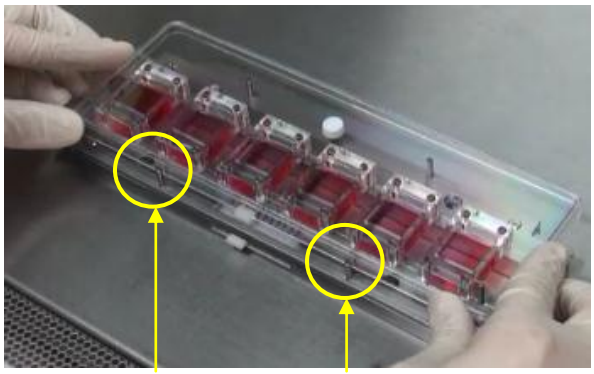
4. Place the chamber holder in a sterile biological safety cabinet. Select the desired stretch ratio using the Stretch Ratio Adjuster. Refer to Stretch Programs for details.

5. To load the seeded stretch chambers onto the chamber holder, turn Offset Screw COUNTER CLOCKWISE, to release tension and allow the chambers to easily slide on and off the metal pins. Once 6 chambers are all set, turn the Offset Screw CLOCKWISE to put holding tension on the chambers.



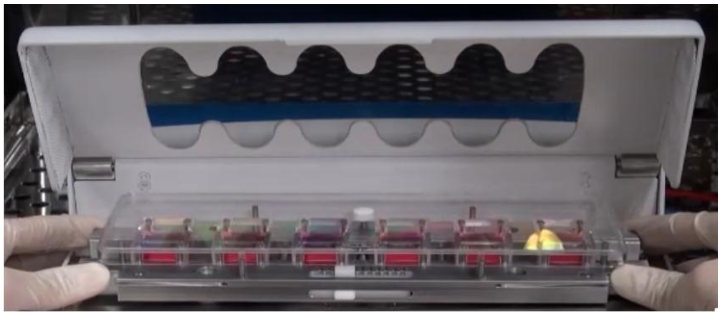


6. Use the plastic square dish (lid or bottom) to protect the chambers. The lid is held in place by the 4 fixed pins toward the bottom edge of the chamber holder. Push the edge of the lid between the 4 pins.



4 fixed pins to hold plastic dish

7. Load the chamber holder back into the Main Unit.



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8. The entire Main Unit can be placed in a cell culture incubator during use. THE MAIN UNIT CAN BE PLACE IN INCUBATORS WITH TEMPERATURES RANGING FROM 10-40°C AT 95% HUMIDITY. THE CONTROLLER SHOULD REMAIN ON THE OUTSIDE OF THE INCUBATOR IN A DRY, CLEAN ENVIRONMENT. THE AIR COMPRESSOR SHOULD BE OPERATED IN A DRY, CLEAN ENVIRONMENT AND ON A LEVEL AND STRONG PLATFORM (OR FLOOR)

6. Coating and Seeding Chambers

1. Stretch Chambers are disposable and are sold ready to use. They are ethylene oxide gas sterilized.

2. Chambers should be coated with an extracellular matrix, like fibronectin, prior to seeding with cells.

   Example coating procedure:
   - A. Dilute fibronectin to a final concentration of 2-20μg/mL in Phosphate Buffered Saline (PBS).
   - B. Place the stretch chambers on the square plastic dish and pour fibronectin solution into the stretch chambers so that the bottom of the chamber is covered with fibronectin solution.
   - C. Incubate at 37°C for at least 30 minutes or up to 2 hours.
   - D. Aspirate the fibronectin solution. The chambers are ready to seed with cells.

3. Coated chambers are ready to be seeded with cells. Incubate the seeded chambers overnight. The next day, check cells for attachment to the chamber.

4. Place the chambers into ShellPa. Allow the cells to stretch a few minutes. Check the cells to confirm cell attachment is maintained.

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7. Stretch Programs

Main Unit

Stretch Ratio Adjuster	% Stretch	Distance
Lock	0%	0 mm
2	2%	0.4 mm
4	4%	0.8 mm
5	5%	1.0 mm
6	6%	1.2 mm
8	8%	1.6 mm
10	10%	2.0 mm
12	12%	2.4 mm
15	15%	3.0 mm
20	20%	4.0 mm

Controller

Program	Knob Function
Run	Toggles between time of current run and total time
Stop	Adjust frequency
	Frequency
Default	60 cycles/minute
Range	1 - 120 cycles/minute
Pattern	Square

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8. **Trouble Shooting:**

If system is not stretching, check the following items:

All tubings and cables connected
Air valve cock open
First air pressure regulator valve is open
Both the first and second air pressure regulators reading the correct pressure
Both the Controller and air compressor are turned on and plugged into an appropriate power source. Power source on
Stretch Ratio Adjuster is not in the “lock” position (0% stretch)

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Warranty

Model No NNMS001

1. The warranty is for **one year**, commencing the date the customer receives the product and includes the instrument casing, and non-wearable parts. The stretch chambers are considered consumables and are only replaced if received damaged or found defective.

2. The warranty does not cover damage to the instrument that is a result of the following circumstances:
   ① Damage caused by dropping, or other impact.
   ② Damage caused by inappropriate operation of the instrument.
   ③ Damage resulting from an attempted repair or modification of the instrument by the user.
   ④ Damage caused by unavoidable external causes such as earthquakes, lightening, fire, flood, gas leak, power surges, or other acts of providence.

3. B-Bridge International, Inc. is free from any responsibility for effects or loss or damages arising from the result of the machine operation.

This warranty assures that B-Bridge International, Inc. will repair our product free of charge as stipulated in our warranty policy. Any shipping charges will be born by buyer.

B-Bridge International, Inc.
20813 Stevens Creek Blvd., Suite 200
Cupertino, CA 95014, USA
Phone: +1-408-252-6200 Fax: +1-408-252-6220
email: customersupport@b-bridge.com

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