

PEG-Maleimide Hydrogel Mechanical Testing (CAT)

Testing Method:

Contact Adhesion Testing (Dr. Al Crosby lab)

→ Contact info:

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** If you plan to run experiments in early morning / at night / during weekends, you should contact to Chris Barney first – ask him if he can let you in at those times.*

** There is another method – indentation – but this machine has only one probe, which does not fit to the size of 10uL hydrogels. Thus, contact adhesion testing method is needed to measure stiffness of 10uL hydrogels precisely.*

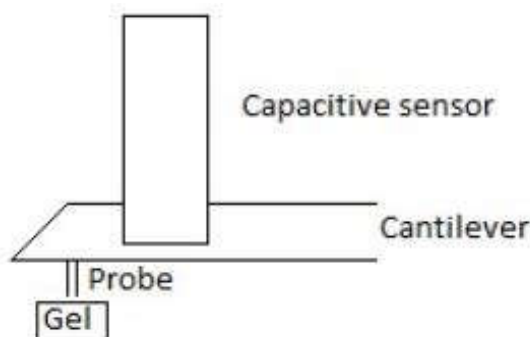
Materials:

- **Flat punches:** cylindrical probes with flat base

(Diameter = 0.5 mm; use the smallest one, but 1mm one can be used)

** Probe size: dependent on height of the hydrogel.*

- **Cantilevers:** #2 (1/128 inch in thickness) works best for PEG-Maleimide hydrogels, but #3 can be used as well.



(Courtesy to Aritra)

[A]. Preparation:

1. Make PEG-Maleimide hydrogels with desired conditions
 - a. Make one gel on one petri dish (use the smallest one: 60*15mm)
 - b. Add ~5mL of 1X PBS to cover the hydrogel: this keeps the gel hydrated.
2. Bring the gels to Conte on the next day: 3rd floor, there is a room where CAT machine is.

[B]. CAT Machine Calibration:

1. Switch on the controller of the Nano-positioner.
2. Plug in the black cord to the signal conditioner.
3. Run the program "USB_6008_DAQ".
4. Click on the white arrow [→] on the screen.
5. Initially, at no deflection, voltage is maximum. Check for minimum and maximum voltages.
6. Earth the cantilever with golden wire.
7. Run the program "SpringCalib".
8. Click on the white arrow on the screen.

9. Set the weight at zero and mark the voltage at 0mg (when no weights are placed on the cantilever).
10. Change the weight with 3-4 different weights and note the voltages.
 - a. Use tweezers
 - b. Weights: 0, 20mg, 50mg, 100mg, 200mg
11. Note the V/g value.
 - a. The value can be calculated with 'LINEST' function on Excel or Google Spreadsheet.
 - b. The typical V/g (Voltage over mass) values are:

Cantilever #	V/g value
2	~ 16
3	~ 2.1
4	~ 0.3

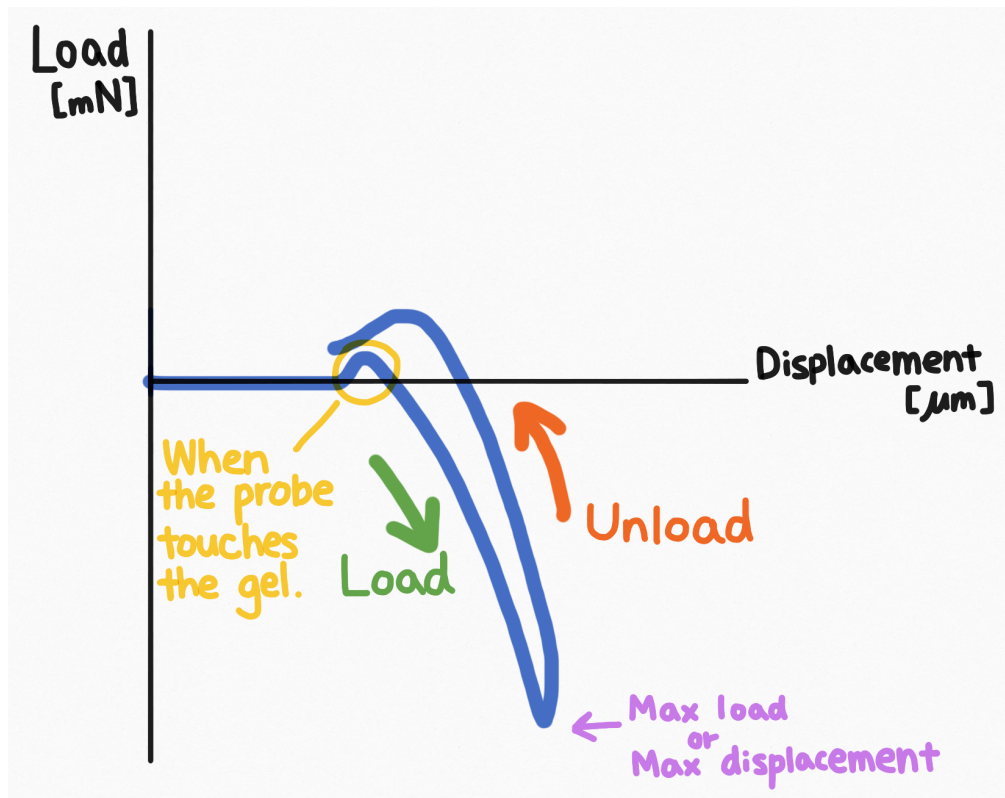
12. Hit red "Stop" button in both the programs.

** In order to find the flat surface of the hydrogel, microscope can be used prior to this calibration step (step [B]).*

[C]. Mechanical Testing on CAT:

1. Attach probe to the cantilever.
2. Run the program named "Contact Adhesion Test".
3. Set the methods on the screen:
 - a. Stopping condition: Maximum load. (Start with a small force: 5~10mN)
 - b. Return to initial Position: Yes
 - c. Load/unload speed: 20 $\mu\text{m/s}$ (set a range between 10~50 $\mu\text{m/s}$)
 - d. Maximum load: 10 mN
 - e. Mass conversion (V/g): put the value that is calculated from the Calibration step (should be around ~16 for cantilever #2)
 - f. Leave everything else blank
4. Place the sample (hydrogel) under the probe, and aspirate PBS from the petri dish.
5. Adjust the probe position.
 - a. Hit "+" under axis 1 on the remote control to lower the probe: move the probe as close as possible to the sample.
 - b. To change the speed of the Nano-positioner, hit "Edit" on the controller. When cursor moves to unit's position, delete or put zeros and hit "Save".
6. Click on the white arrow [$\rightarrow$], and hit "Start" button on the screen.
7. After measuring the sample, move the sample (hydrogel) away from the probe: Place the probe above the bottom of the petri dish (where there is no gel) in order to account for the deflection of the cantilever so that when the run is started it touches the bottom surface.
 - a. This is called "glass run" and it is needed to measure the height of hydrogel. Heights are needed for stiffness calculation later.
8. Set "Load speed/unload speed: 100 $\mu\text{m/s}$ ".
9. Set "Maximum load: 10 mN (for glassrun)".
10. Click on the white arrow, then hit "Start".

Expected graph on screen (Contact Adhesion Test program on the computer):



- 1) The graph for the “gel runs” should look like the figure above.
- 2) The graph for the “glass runs” should look similar to this, but it has straight lines for both loading and unloading part.

* Graphs for “gel runs” have *slightly curved lines* due to the heterogeneity of hydrogels.