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PhotoHA®-Soft Rheology Study

Abstract: *The following study was conducted to assess the rheological behavior of PhotoHA®-Soft at different concentrations using the Elastosens rheometer. PhotoHA®-Soft (PH) powder was reconstituted at 1%, 2%, 3%, 4% and mixed with LAP photoinitiator. Samples were cured under 100% intensity exposure of 405 nm UV light for 10 minutes. Results show that at 0.034% LAP concentration, as the concentration of PH increases, the maximum shear storage modulus increases as well as the rate of photocrosslinking.*

Materials

Name/Description	Part Number	Lot Number	Degree of Methacrylation
PhotoHA®-Soft	5388	8958-A	29%
LAP	85073-19-4	BRCLAP-I-0324-01	-
DPBS	21600-044 1x50 L	2814720	-

Results

Figure 1 below shows the rheological curves of PhotoHA®-Soft (PH) at 1%, 2%, 3%, and 4% concentrations, and a fixed LAP concentration of 0.034%. The vertical dotted lines at minutes 5 and 15 represent the start and end of UV photostimulation respectively at 100 % intensity. The graph shows that as the concentration of PH increases, the maximum shear storage modulus (G'), or rigidity of the material, increases as well as the rate of photocrosslinking.

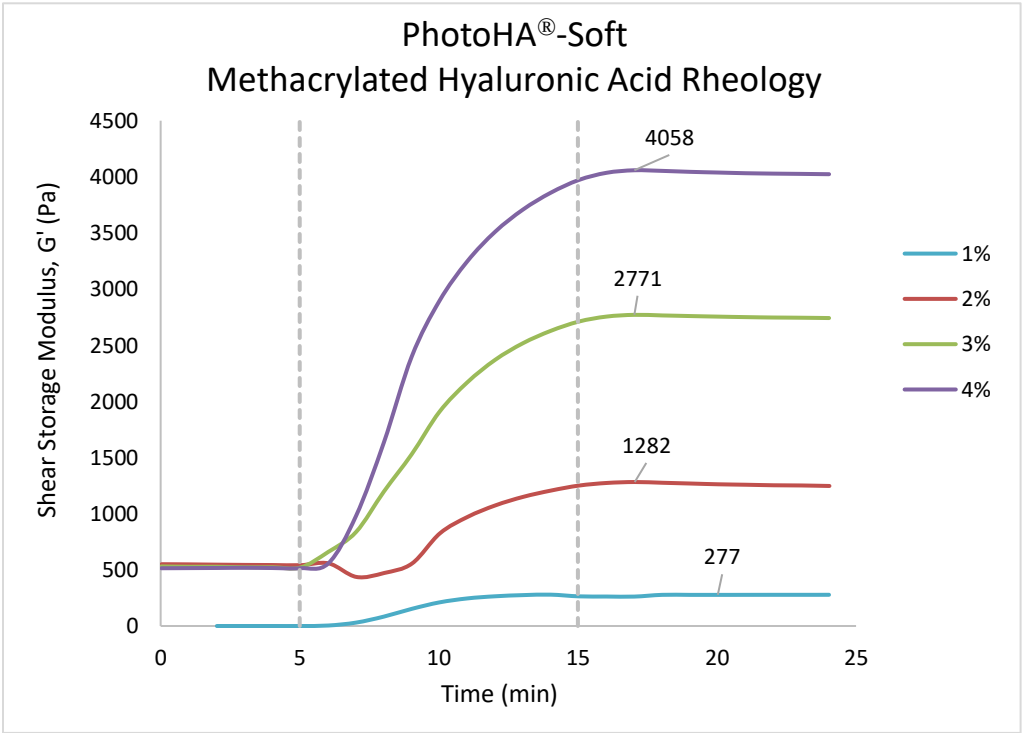

Figure 1. Compilation of viscoelastic curves of photocrosslinked PhotoHA®-Soft at concentrations 1%, 2%, 3%, and 4% with 0.034% LAP. Dotted lines indicate the start and end of 405 nm UV light photostimulation.

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Conclusion

In conclusion, the ability to reconstitute PhotoHA® at different concentrations allows for the potential to fine-tune the final photocrosslinked hydrogel stiffness for various applications. Manipulating other variables such as photoinitator concentration, temperature, time of exposure, and UV light intensity can potentially provide further variations and fine-tunability to produce a desired hydrogel with distinct rheological properties.

Procedure

The following sample preparation and experimental set up procedures were performed to carry out the study. Briefly, lyophilized PhotoHA® was reconstituted at 1%, 2%, 3%, and 4% with 1X PBS. Following Advanced Biomatrix's standard preparation protocol, a solution of LAP at 17 mg/ml was added to dissolved PhotoHA® to achieve a final LAP concentration of 0.034% (see *"Sample Preparation"* procedure below). Then, the Elastosens rheometer was calibrated, and the experimental parameters set (see *"Elastosens Experimental Set Up"* procedure below). Each experimental group was tested in duplicate sequentially and the test data averaged. The same bulk components, i.e. PhotoHA®, 1X PBS, and LAP were used for all test groups in this study.

Sample Preparation

1. The corresponding volume of 1X PBS was added into individual vials of lyophilized PhotoHA® to yield concentrations of 1%, 2%, 3%, and 4%. They were then gently mixed on a shaker at 2-8°C until fully dissolved.
2. LAP powder was solubilized at 17 mg/ml in 1X PBS, sterile filtered, and covered in aluminum foil to minimize light exposure.
3. The calculated volume of LAP solution was added to each PhotoHA® vial (0.02x of solubilized PhotoHA® volume) and further mixed until homogeneous.
4. All samples were stored at 2-8°C aseptically prior to testing.

Elastosens Experimental Set Up

1. The Elastosens was turned on and calibrated once via vibration calibration.
2. The temperature was set to 20°C using the manual temperature control.
3. Once the temperature was equilibrated, a large empty sample cup was inserted in the testing chamber and secured. The same sample cup was used for all test groups in this study.
4. New sample files were created for each group in the following folder: PhotoHA Soft.
    a. File format: product name, lot number, concentration.
        i. E.g. PhotoHA Soft 8958-A 1%.

5. The following test parameters were set for all test conditions except for PhotoHA® at 1%:
    a. Type – Stiff.
    b. Sample File – Named as noted above.
    c. Test Name – Tester's initials followed by the number of the test run under the same file name (each test was run in duplicate, and the resulting curves averaged).
        i. E.g. the first test ts01, second test ts02.
    d. Custom Information:
        i. Volume – 4 g.
        ii. Oil – No.
        iii. Concentration – Varies.
        iv. Photoinitiator – LAP.
        v. Photoinitiator concentration – 0.034%.

vi. Cup size – Large.
vii. Light intensity – 100%
viii. Exposure time – 10 min.
ix. Temperature – 20°C.

6. For PhotoHA® 1%, all test parameters outlined in step 5 remained constant except for the sample type being tested, which was set as “Soft” instead of “Stiff”.
7. The following test sequences were set in the “Measurement Sequences” window for all samples:
    a. Sequence 1: Thermal incubation
        i. Duration: 5 min.
        ii. Step: 1 min.
        iii. Temperature configuration: manual.
    b. Sequence 2: Photocrosslinking
        i. Duration: 10 min.
        ii. Step: 1 min.
        iii. Temperature configuration: manual.
        iv. Photostimulation LED 405nm: 100%.
    c. Sequence 3: Equilibration
        i. Duration: 10 min.
        ii. Step: 1 min.
        iii. Temperature configuration: manual.

8. The sample cup calibrated once prior to testing each group.
9. The sample cup was removed from the machine and placed on a scale to add 4 g ± 0.1 g of sample.
10. The sample-containing cup was placed into the machine and the test started.
11. After the first test was finished, a second test (following the procedure above) was run to obtain a duplicate test under the same file name.
12. The sample was removed from the cup after gelled, and the cup rinsed with milli-Q water and dried to be reused.