ADVANCED BIOMATRIX
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PhotoGel® 50% DoM and PhotoHA®-Stiff Combination Rheology

Abstract: *Being able to combine methacrylated gelatin and methacrylated hyaluronic acid is of key interest for scientists focused on tissue engineering and 3D bioprinting, as these two materials help generate an environment that more closely resembles an in vivo environment.*

The following study was conducted to assess the rheological effects of combining PhotoGel® with a 45% degree of methacrylation (PG) and PhotoHA®-Stiff (PH) at different concentrations. Each experimental group consisted of equal parts PhotoGel® at 5% and 10%, and PhotoHA®-Stiff at 1%, 2%, 3%, or 4% concentration. Two different test parameters were used to assess the effects of combining materials on PhotoGel® and PhotoHA® to help reinforce the data and results. The first set of tests utilized a 10-minute exposure at 100% UV intensity. The second set used a 5-minute exposure at 15% intensity. Results show that the addition of high concentrations of PhotoHA® significantly increased the rheological properties of PhotoGel®. The data for 5% and 10% PhotoGel® combined with 4% PhotoHA® is not shown since high and prolonged UV exposure of 4% PhotoHA® caused the hydrogel to generate inaccurate data, potentially due to shrinking, dehydration, or sample detachment.

Materials

Name/Description	Part Number	Lot Number	Degree of Methacrylation
PhotoGel® 50% DoM	VL3500000502	9530	45%
PhotoHA®-Stiff	5212	9724	74%
DPBS	21600-044 1x50 L	2814720	-
LAP	5269-100MG	-	-

Results

Figure 1 below shows the results on the rheological impact of adding various PhotoHA® concentrations to PhotoGel® 5% and 10% using the first protocol stated above.

Figure 2 shows the trends in the maximum stiffness for each material combination.

For PhotoGel® 5%, figure 1A and figure 2 show that the addition of PhotoHA® increases the gel stiffness. The higher the concentration of PhotoHA®, the higher the stiffness. The slight drop in stiffness for 5% PhotoGel® + 3% PhotoHA® post crosslinking in figure 1A is believed to be due to the sample shrinking or dehydration and sample detachment caused by long and intense UV exposure. Data for added PhotoHA® at 4% was not included due to similar issues.

Figure 1B and figure 2 show that a slight decrease in stiffness is seen when introducing 1% PhotoHA® to 10% PhotoGel®, yet the stiffness increases with the addition of 2% and 3% PhotoHA®.

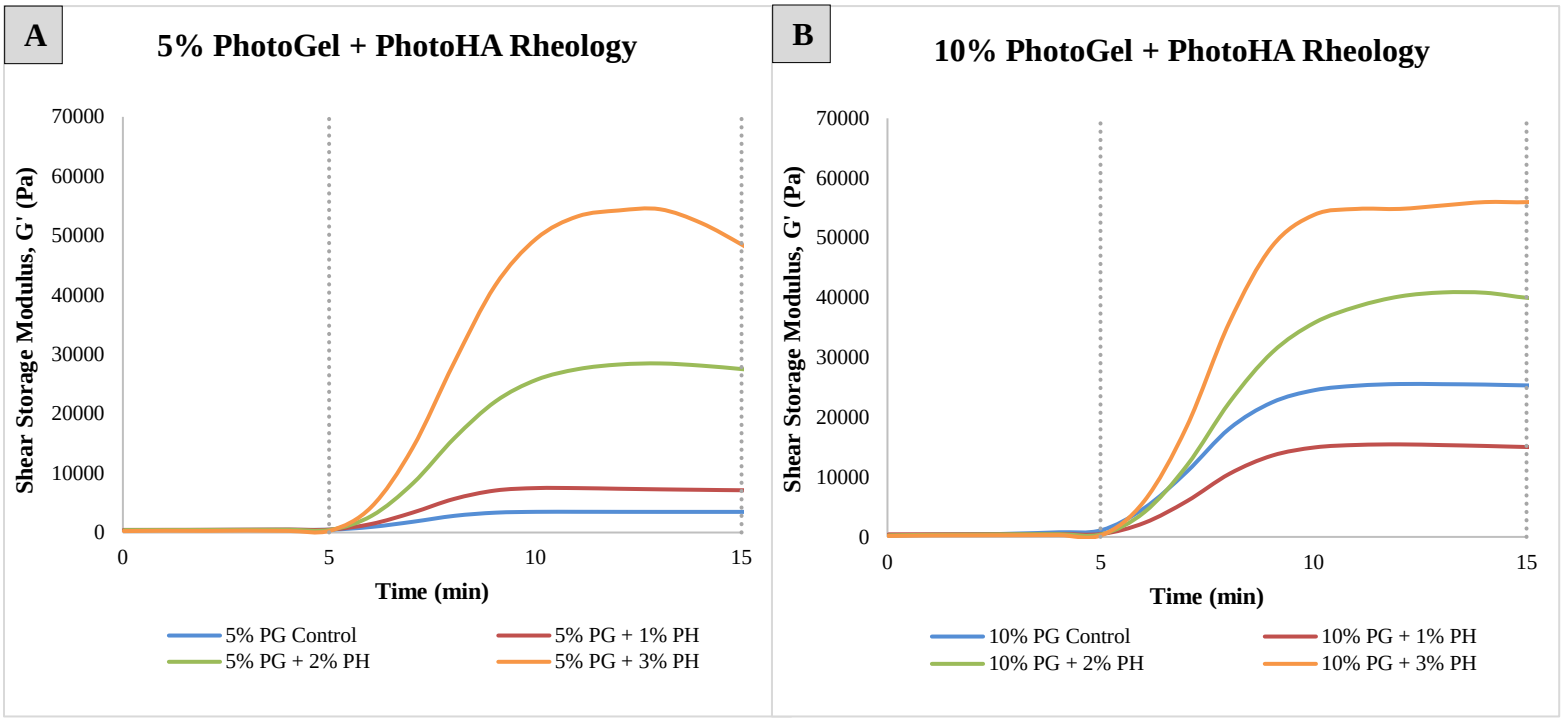

Figure 1. Combination Rheology of PhotoGel® 5% (A), 10% (B) and PhotoHA® 1%, 2%, 3% using PhotoGel® test parameters: 10-minute exposure of 100% UV light intensity. Dotted lines indicate the start and end of UV photostimulation.

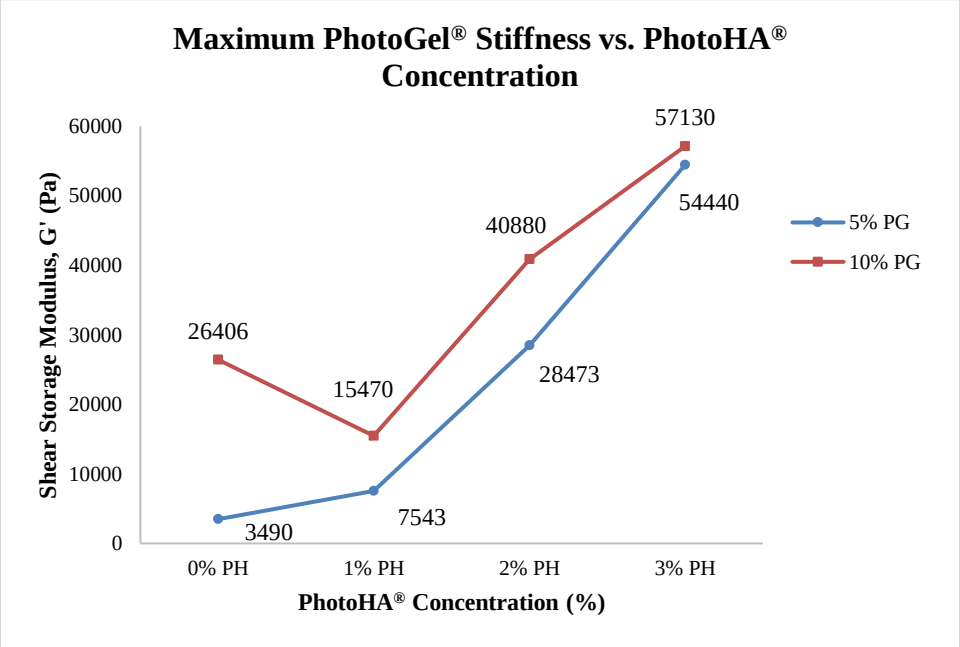

Figure 2. Trend graph of maximum stiffness of PhotoGel® 5% and 10% with 1%, 2%, and 3% PhotoHA®.

Figure 3 shows the results on the rheological impact of adding 5% and 10% PhotoGel® to 1%, 2%, 3%, and 4% PhotoHA® using the second test protocol outlined above.

Figure 4 shows the trends in maximum stiffnesses of the combined materials.

For all PhotoHA® concentrations, adding 5% PhotoGel® decreases the maximum gel stiffness, while adding 10% PhotoGel® results in a higher stiffness compared to the 5% PhotoGel® combinations.

However, for 3% and 4% PhotoHA®, adding 10% PhotoGel® results in a lower stiffness when compared to their controls (0% PhotoGel®). At these high PhotoHA® concentrations, the photocrosslinked gels are much stiffer than pure 5% and 10% PhotoGel®; hence a drop in G' is seen when introducing 5% and 10% PhotoGel®. For lower PhotoHA® concentrations of 1% and 2%, adding 10% PhotoGel® resulted in higher gel stiffnesses compared to their controls (0% PhotoGel®).

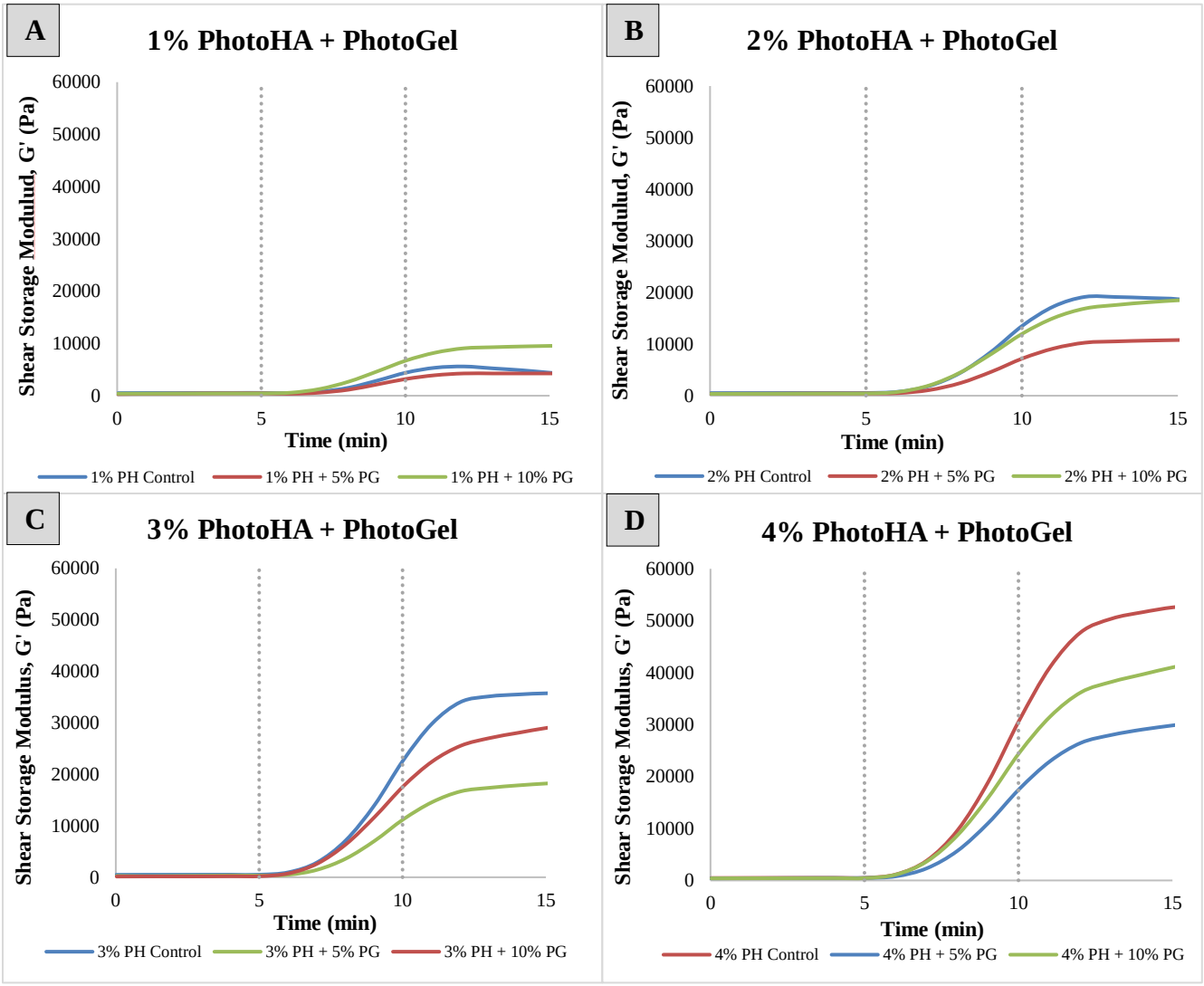

Figure 3. Combination Rheology of PhotoHA® 1% (A), 2% (B), 3% (C), 4% (D) and PhotoGel® 5%, 10% using PhotoHA® test parameters: 5-minute exposure of 15% UV light intensity. Dotted lines indicate the start and end of UV photostimulation.

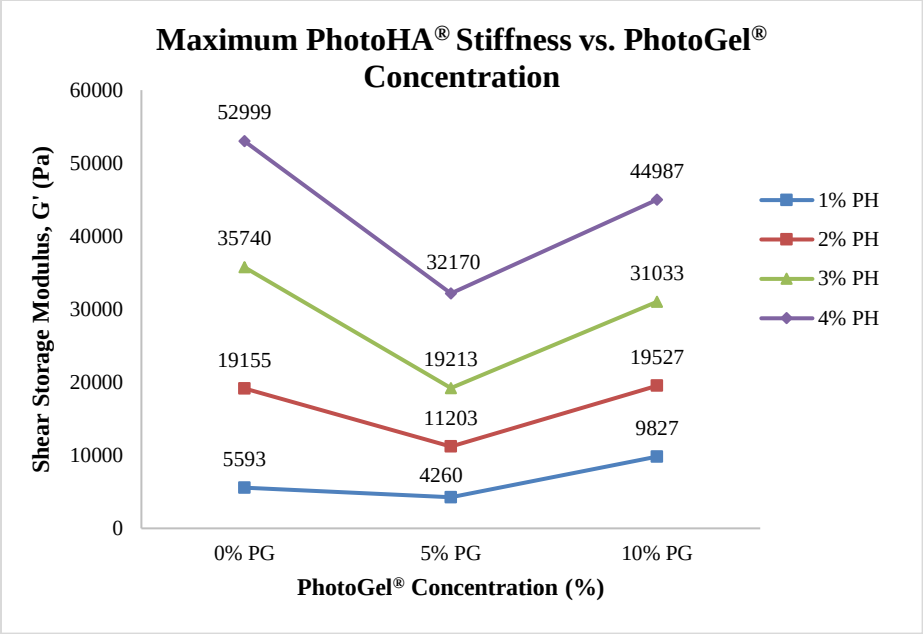

Figure 4. Trend graph of maximum stiffness of PhotoHA® 1%, 2%, and 3% with 5%, and 10% PhotoGel®.

Conclusion

In conclusion, several PhotoGel® 50% DoM and PhotoHA®-Stiff combinations can be used to generate a wide range of gel stiffnesses and more closely replicate *in vivo* like tissues and cellular environments for bioprinting and tissue engineering.

Overall, pure PhotoHA® achieved a higher gel stiffness after photocrosslinking compared to pure PhotoGel® 5%. Thus, adding PhotoGel® 5% to any PhotoHA® concentration (1% or greater) decreased the gel stiffness. Adding PhotoGel® 10% to PhotoHA® resulted in a higher stiffness than adding 5% PhotoGel®, however it decreased the stiffness compared to pure PhotoHA® samples (2% or greater).

The test does not look at flexibility, brittleness, hydrophobicity, viscosity, or other similar important parameters in hydrogels, bioprinting or tissue engineering that are affected by mixing various combinations of materials together. Gelatin and Hyaluronic Acid are both known for having unique properties, and these variables should also be considered during experimental set-up.

Procedure

The following sample preparation and experimental set up procedures were performed to carry out the study. Briefly, a bulk solution of high concentration PhotoGel® was diluted to 5% and 10% with 1X PBS. Lyophilized PhotoHA® was reconstituted at 4% and diluted to 1%, 2%, and 3% with 1X PBS (see *"Sample Preparations"* procedure below). Then, the Elastosens was calibrated, and the experimental parameters set (see *"Elastosens Experimental Set Up"* procedure below). Since PhotoGel® and PhotoHA® respond differently to different test protocols, tests were run using two different parameters: a 5-minute exposure of 15% UV intensity (PhotoHA® standard) and a 10-minute exposure of 100% UV intensity (PhotoGel® standard). Each experimental group was then tested in duplicate sequentially and the test data collected and processed. The same bulk components, i.e. PhotoGel®, PhotoHA®, and 1X PBS were used for all test groups in the study.

Sample Preparations

1. For PhotoGel®, the bulk solution was incubated at 40°C to liquefy.
2. The corresponding amount of 12.9% solution was aliquoted into individual tubes and kept at 40°C.
3. The corresponding amount of warm 1X PBS was added into each tube to yield concentrations of 5% and 10%. Solutions were mixed by gently inverting the tubes back and forth.
4. For PhotoHA®, 1X PBS was added into individual vials of lyophilized material to yield a 4% concentration and incubated at 2-8°C on a shaker to mix gently until fully dissolved.
5. All 4% solutions of PhotoHA® were combined and further mixed to ensure homogeneity. The bulk solution was then aliquoted into individual tubes and kept at 2-8°C.
6. The corresponding amount of 1X PBS was added into each tube to yield concentrations of 1%, 2%, and 3%. The solutions were left to mix on a shaker table.
7. All samples were covered in aluminum foil to minimize light exposure and incubated at 40°C (for PhotoGel®) and 2-8°C (for PhotoHA®) 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: Combination Materials.
    a. File format: concentration and product initials.
        i. E.g.: 5% PhotoGel® + 1% PhotoHA®
        ii. E.g.: 10% PhotoGel® + 3% PhotoHA®

5. The following test parameters were set for all test conditions:
    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. Ex - the first test ts01, second test ts02.
    d. Custom Information:
        i. Volume – 2 g.
        ii. Oil – No.
        iii. Concentration – Varies.
        iv. Photoinitiator – LAP.
        v. Photoinitiator concentration – 0.25%.
        vi. Cup size – Large.
        vii. Light intensity – 15% or 100%
        viii. Exposure time – 5 min or 10 min.
        ix. Temperature – 20°C.

6. The following test sequences were set in the "Measurement Sequences" window:
    a. Sequence 1: Thermal incubation
        i. Duration: 5 min.
        ii. Step: 1 min.
        iii. Temperature configuration: manual.
    b. Sequence 2: Photocrosslinking

i. Duration: 5 or 10 min.
ii. Step: 1 min.
iii. Temperature configuration: manual.
iv. Photostimulation LED 405nm: 15% or 100%.

c. Sequence 3: Equilibration
i. Duration: 10 min.
ii. Step: 1 min.
iii. Temperature configuration: manual.

7. The sample cup was calibrated once prior to testing each group.
8. The sample cup was removed from the machine and placed on a scale to add 2 g ± 0.1 g of sample.
9. The sample-containing cup was placed into the machine and the test started.
10. 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.
11. The sample was removed from the cup after gelled, and the cup rinsed with milli-Q water and dried to be reused.