Photochemical Modulation of Hydrogel Scaffolds and Nanoindenter-based Stiffness Quantification

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CHANL — MAKE NANO – MEASURE NANO

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Introduction

- The extracellular matrix (ECM) is an essential part of the cellular microenvironment and conveys both chemical and mechanical cues that play a significant role in regulating cellular behavior[1,2,3].
- Biomechanical properties of the ECM, such as stiffness and porosity, are known to dictate the forces exerted by cells onto their surroundings and influence cellular function in complex, three-dimensional (3D) environments *in vivo*[1,2,3].
- Light-based stiffness modulation can be achieved using a photosensitizer (PS), a molecule that absorbs visible light and reaches an excited triplet state[2,4].
- This longer-lived triplet state of a PS produces reactive species such as singlet oxygen that fuel the necessary biomolecular reactions to either increase or decrease stiffness depending on the type of PS and its corresponding excitation wavelength[2,4].

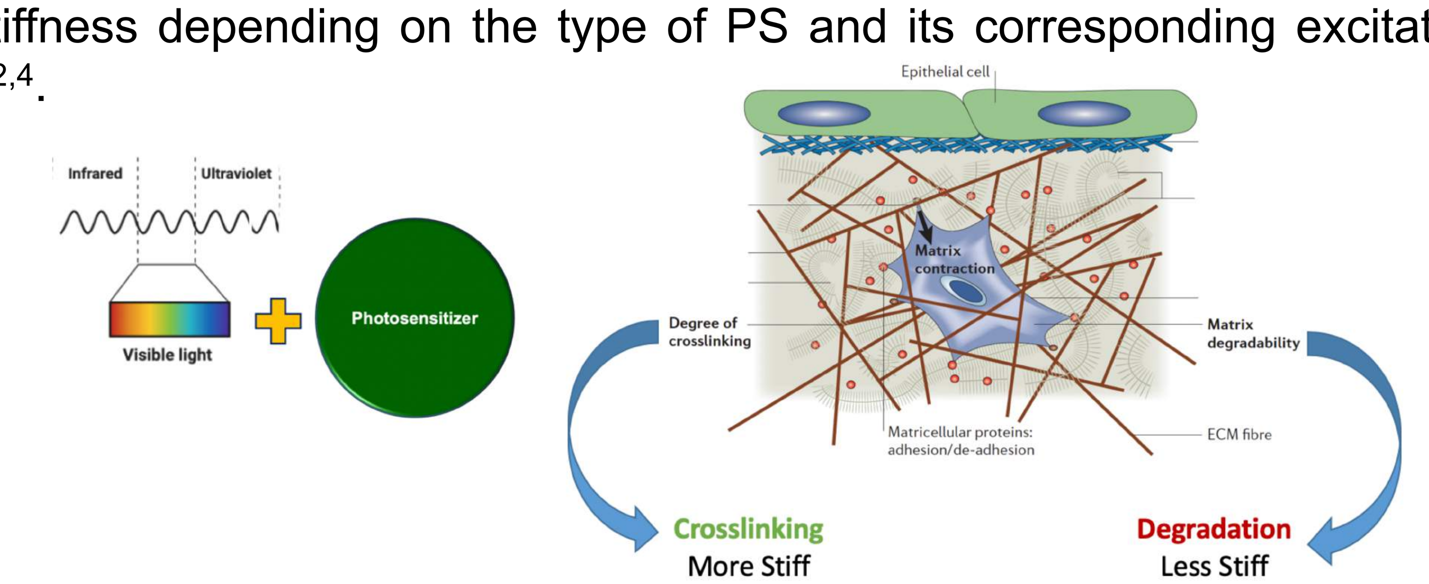
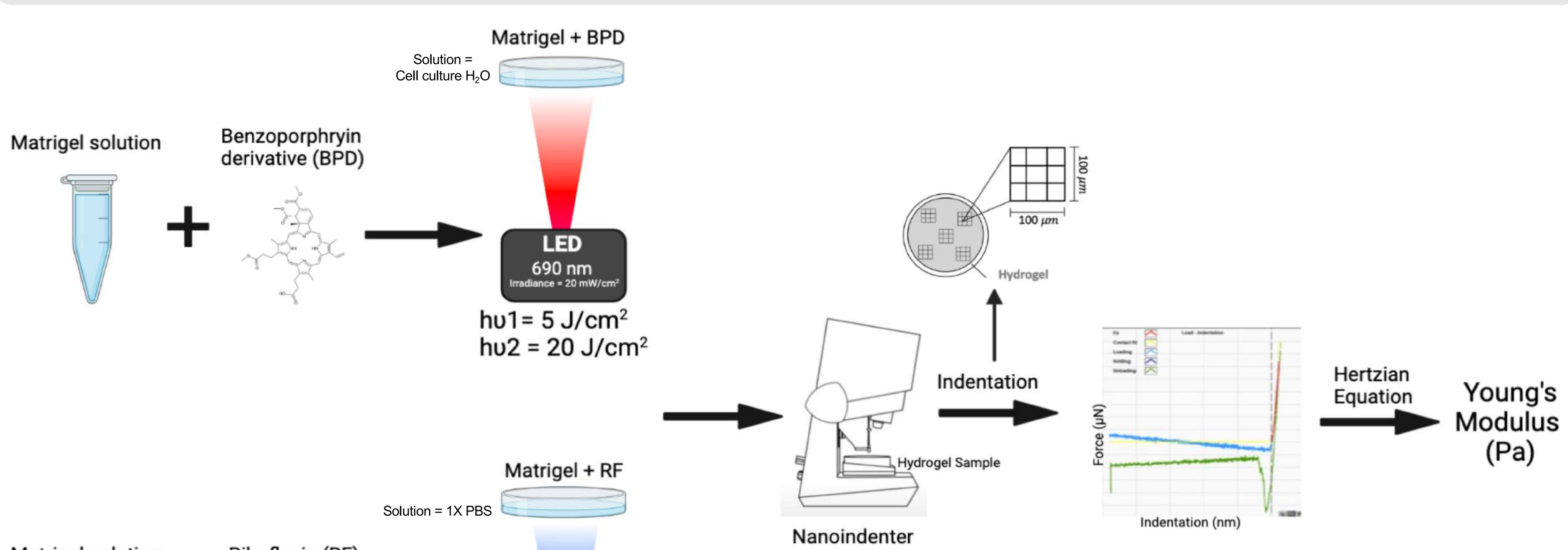


Figure 1[3]**.** Altering the mechanical properties of the cellular microenvironment using photochemistry

Aim 1: Quantify the effects of benzoporphyrin derivative (BPD)-mediated photodegradation and riboflavin-mediated photocrosslinking on the stiffness of Matrigel hydrogels using nanoindentation.
Aim 2: Develop a photomask prototype to serve as a preliminary optical patterning platform.

Methods



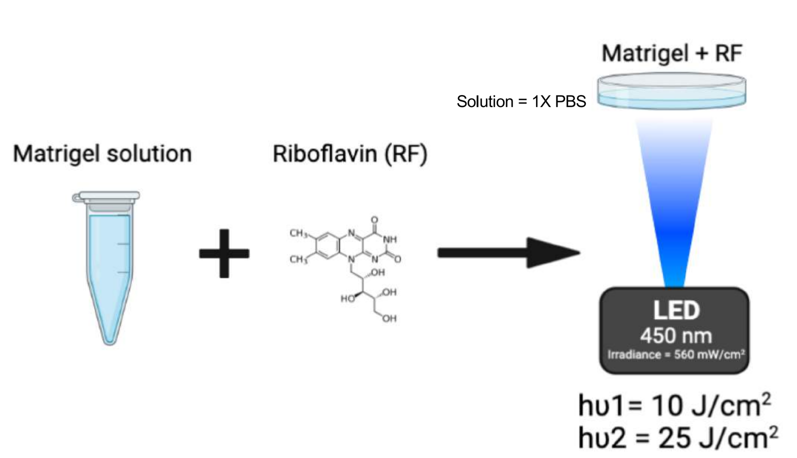
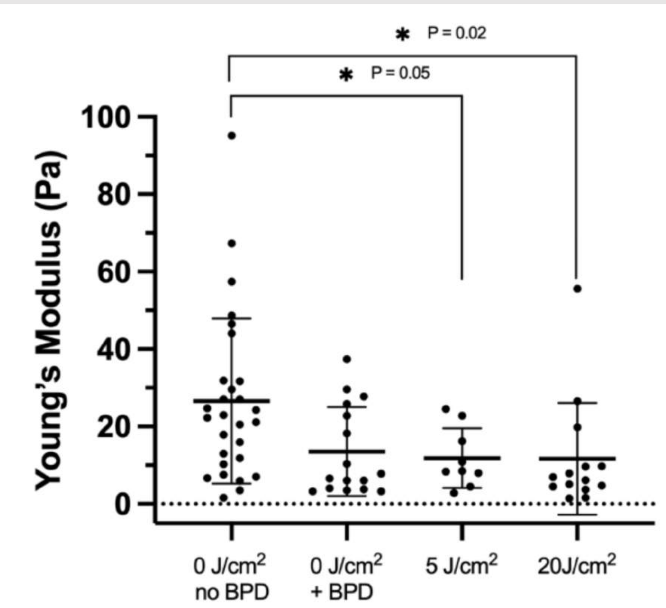


Figure 2. Experimental setup including two different light sources and photosensitizers followed by stiffness quantification using nanoindentation.
Figure created in BioRender.

- For experiments with BPD, the final concentration of PS was **250 nM** and Matrigel hydrogels were incubated for **45 minutes** before irradiation and nanoindentation.
- For experiments with RF, the final concentration of PS was **0.1%** and Matrigel hydrogels were incubated for **24 hours** before irradiation and nanoindentation using a heated stage.
- Force curves were analyzed with Piuma Dataviewer V2.3 software.

Results

BPD-mediated Photodegradation



Gel Condition	Number of Curves Fit/Total Curves*	Mean (Pa)	SD
60% Matrigel No BPD no $h\nu$	28/45	26.56	21.30
60% Matrigel BPD no $h\nu$	16/45	13.52	11.52
60% Matrigel BPD $h\nu 1$	9/45	11.83	7.712
60% Matrigel BPD $h\nu 2$	14/45	11.68	14.43

*Curves fit using Hertz model and R^2 threshold of 0.8

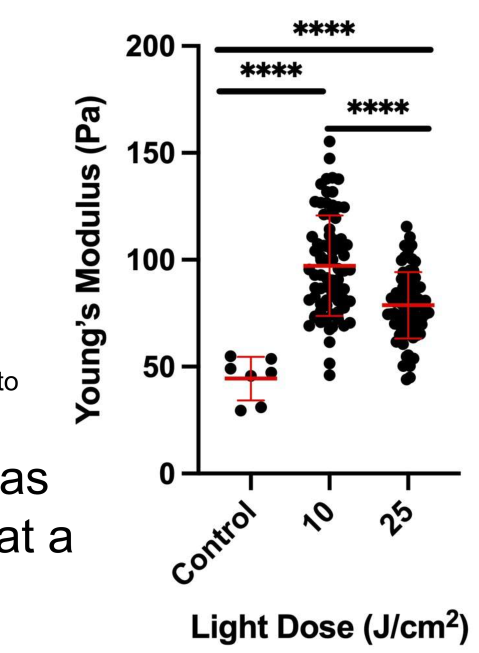
Figure 3. Distribution of stiffness measurements (recorded as Young's modulus) collected for each hydrogel.. * signifies p ≤ 0.05, according to an unpaired, two-tailed t-test.

- The lowest average stiffness recorded was 11.68 Pa when using red light (690 nm) at a light dose of 20 J/cm².
- There was a significant difference (P≤0.05) between the average stiffness of the control group that did not receive any light and or PS, and the two groups that received light doses of 5 J/cm² and 20 J/cm².

RF-mediated Photocrosslinking

Gel Condition	Number of Curves Above R^2 = 0.95	Mean (Pa)	SD
0 J/cm²	7/45	44.44	10.23
10 J/cm²	75/80	97.24	23.53
25 J/cm²	75/80	78.75	15.53

Figure 4. Distribution of stiffness measurements (recorded as Young's modulus) collected for each hydrogel. **** signifies p < 0.0001, according to an unpaired, two-tailed t-test.



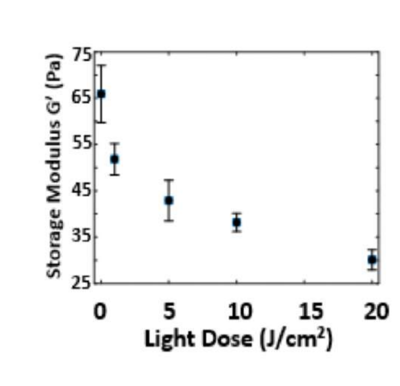
- The highest average stiffness recorded was 97.24 Pa when using blue light (450 nm) at a light dose of 10 J/cm²
- Although there was a limited amount of stiffness measurements for the control group that received no light and no PS, there was a significant difference (P≤0.05) between the average stiffness of the control and the two groups that received light doses of 10 J/cm² and 25 J/cm².

Figure 5. CAD drawing and 3D printed version of photomask platform.

- A photomask platform was developed using computer aided design software (OnShape) and 3D printed using a Stratasys printer
- The dimensions are 2.62 in x 2.62 in x 0.270 in and it is designed to fit a 35 mm glass bottom dish with a 10 mm microwell.

Discussion and Conclusion

BPD-mediated Photodegradation



Shear Rheology		Nanoindentation	
Celli Lab Light Dose J/cm²	Approximate Celli Lab Stiffness in Young's Modulus (Pa)	My Light Dose J/cm²	My Average Stiffness in Young's Modulus (Pa)
0	195	0 (60% Matrigel, no BPD, no hv)	26.56
5	156	5	11.83
20	90	20	11.68

$E = 2G'(1+\mu)$
Where E = Young's Modulus (Pa)
G' = Storage modulus (Pa)
μ = 0.5 (assumed Poisson's ratio for all samples in both labs)

Figure 6. Comparison of stiffness measurements taken with a shear rheometer and a nanoindenter.

- The large standard deviations observed with BPD-mediated photodegradation (**Fig. 3**) indicate the nanoindenter's poor signal-to-noise ratio due to the compliance of the hydrogel.
- In comparison to stiffness measurements from the Celli Lab (Univ. of Massachusetts at Boston) taken with a shear rheometer, there was a consistent trend of decreasing stiffness for the same hydrogel and BPD concentration as light dose increased.
- The discrepancies between the average stiffness values (**Fig. 6**) is likely due to inherent differences in how the two instruments assess the mechanical properties of a hydrogel.

RF-mediated Photocrosslinking

- Previous work reported stiffness values of approximately 420 and 465 Pa for collagen hydrogels irradiated at light doses of 10 and 25 J/cm[5], respectively.
- The average stiffnesses reported here in Matrigel using the same light doses and riboflavin concentration are more compliant at 97.24 Pa and 78.75 Pa (**Fig. 4**).
- This difference may be dependent on the heterogenous composition of Matrigel and that it is comprised of only ~30% collagen, of which light-induced crosslinking effects such as stiffening are thought to result primarily from crosslinks in collagen[4,5].

Future Directions

1. Quantify stiffness modulation in Type I collagen hydrogels after RF-mediated photocrosslinking at 450 nm and interrogate concurrent changes in porosity.
2. Conduct preliminary optical patterning experiments and prototype improvements.

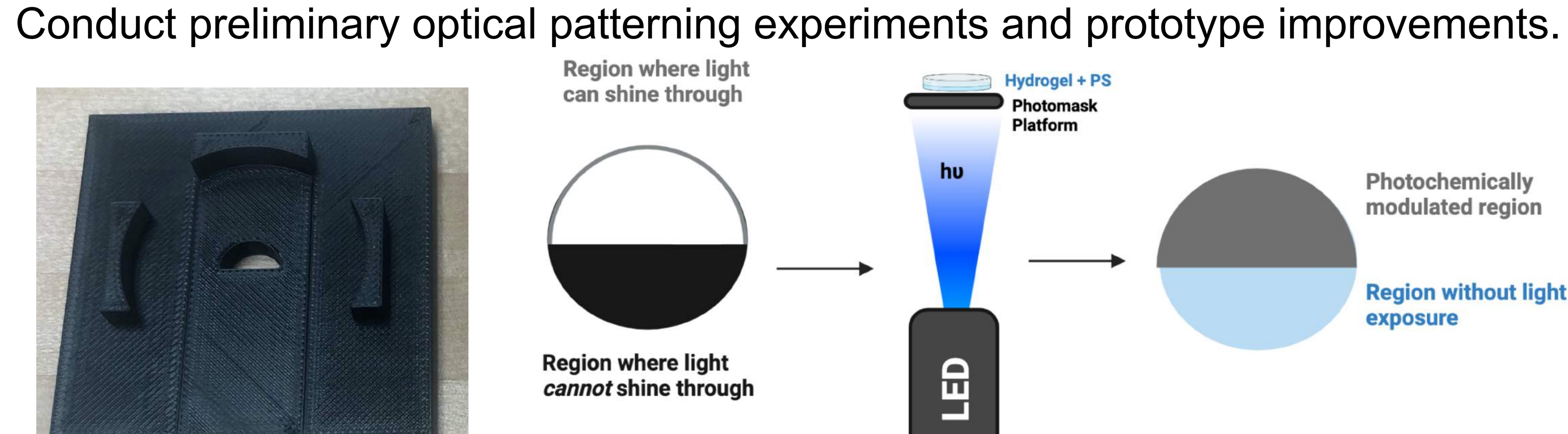


Figure 7. Experimental setup for future work with photochemistry-based optical patterning.

References: [1]Lu, P., Weaver, V. M., & Werb, Z. J Cell Bio. (2012). [2]Sorrin, A.J. et al. Photochem Photobiol (2020). [3] Griffith, L. G., & Swartz, M. A. Nature reviews (2006). [4]Redmond, R. W., & Kochevar, I. E. Photochem Photobiol. (2019). [5]Cramer, G. M., & Celli, J. P. Photochem Photobiol (2020).