

Research Article

UV Dose Governs UV-Polymerized Polyacrylamide Hydrogel Modulus

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Polyacrylamide (PAA) hydrogels have become a widely used tool whose easily tunable mechanical properties, biocompatibility, thermostability, and chemical inertness make them invaluable in many biological applications, such as cell mechanosensitivity studies. Currently, preparation of PAA gels involves mixtures of acrylamide, bisacrylamide, a source of free radicals, and a chemical stabilizer. This method, while generally well accepted, has its drawbacks: long polymerization times, unstable and toxic reagents, and tedious preparation. Alternatively, PAA gels could be made by free radical polymerization (FRP) using ultraviolet (UV) photopolymerization, a method which is quicker, less tedious, and less toxic. Here, we describe a simple strategy based on total UV energy for determining the optimal UV crosslinking conditions that lead to optimal hydrogel modulus.

1. Introduction

In recent years, polyacrylamide (PAA) hydrogels have gained popularity as substrates for cell culture due to their biocompatibility, hydrophilicity, low cost, and ease of preparation, as well as their attractive mechanical properties [1, 2]. Another advantage of PAA hydrogels is the ability to vary the ratio of monomer (acrylamide) to crosslinker (bisacrylamide) and total monomer concentration; these strategies lead to hydrogels with Young's modulus anywhere between 0.1 and 300 kPa and emulate any physiological soft tissue [3]. PAA hydrogels are commonly implemented as cell substrates to assess stiffness-dependent cell behavior due to their tunable compliance. For example, in the seminal paper by Lo et al. [4], the authors used PAA hydrogels of two different moduli to demonstrate that cells preferentially move from a softer substrate to a stiffer substrate, a phenomenon known as durotaxis. Since then, PAA hydrogels of different moduli have been used to demonstrate the role of substrate stiffness on cell morphology and the cytoskeleton [5], cancer cell metastasis and invasiveness [6, 7], cellular responses to drugs and toxins [8, 9], and directing cell phenotype [10].

Currently, when used as cell culture substrates, PAA gels are mainly polymerized with catalysts such as tetramethylenediamine (TEMED) and ammonium persulfate (APS), which are highly toxic and also lead to slow polymerization times—on the order of 45 min to 1 h for lower gel precursor concentrations [1, 11]. Photocrosslinking with various photoinitiators, such as Irgacure 2959, has been more recently employed for the fabrication of PAA hydrogels with a stiffness gradient [1, 10, 12], or for the quick preparation of large PAA hydrogel arrays for applications such as drug screening [8]. Photocrosslinking circumvents the use of toxic catalysts and is typically much faster—on the order of 1–5 min [8]. Final properties of UV-polymerized gels depend on the UV wavelength, uniformity, light intensity, and exposure times [1].

In this study, we present a method for preparing UV-polymerized PAA hydrogels with a focus on UV dose, rather than UV intensity or UV exposure time independently. We demonstrated that, for UV-polymerized hydrogels, rather than independently varying UV exposure time and intensity, one should consider the UV dose: interestingly, for all PAA hydrogels as well as UV parameter combinations tested here,

TABLE 1: Concentrations and volumes of acrylamide and bisacrylamide required to prepare polyacrylamide hydrogels of various moduli.

Nomenclature	Acrylamide concentration (%)	Bisacrylamide concentration (%)	Acrylamide from 40% stock solution (mL)	Bisacrylamide from 2% stock solution (mL)	DI water (mL)
A5-B.025	5	0.025	0.625	0.063	4.31
A5-B.10	5	0.100	0.625	0.250	4.13
A8-B.10	8	0.100	1.000	0.250	3.75
A8-B.25	8	0.250	1.000	1.000	0.63
A12-B.25	12	0.250	1.500	0.625	2.88

the optimal UV dose was $\sim 5 \times 10^3$ mJ/cm². The optimal dose was defined as the lowest dose required to reach hydrogel modulus saturation, above which the modulus did not change appreciably.

2. Materials and Methods

2.1. Materials. Acrylamide (A) (40% w/v), bisacrylamide (B) (2% w/v), and Irgacure 2959 were purchased from BASF Corporation (Florham Park, NJ); phosphate-buffered saline (PBS) and Repel Silane[®] were purchased from GE Healthcare Life Sciences (Logan, UT); and silicone spacers were purchased from Grace Bio-Labs (Bend, OR). RPMI media and fetal bovine serum (FBS) were purchased from GE Healthcare Hyclone (Little Chalfont, UK). Trypsin was purchased from Sigma-Aldrich (St. Louis, MO). MDA-MB-231 breast cancer cells were obtained from NCI DCTD Repository (NCI, Frederick, MD). Type I collagen and sulfosuccinimidyl-6-(4'-azido-2'-nitrophenylamino)hexanoate (Sulfo-SANPAH) were purchased from Thermo Fisher (Waltham, MA), and penicillin-streptomycin (pen/strep) was purchased from MP Biomedicals (Santa Ana, CA).

2.2. Hydrogel Preparation

2.2.1. UV-Polymerized Hydrogels. To prepare the PAA gel precursor solution, A, B, and deionized (DI) water were added at specific ratios (Table 1) to prepare 5 mL working solutions. The gel precursor solution was degassed for 30 min at which point the photoinitiator, Irgacure 2959 for UV-crosslinked gels, was added to the solution at 0.1% w/v final concentration and mixed gently. Irgacure 2959 was chosen because it has been shown to be UV sensitive (at UV wavelength of 365 nm) and also nontoxic to cells at low concentrations [13]. The solution was then pipetted on the center of a glass slide that was pretreated with Repel Silane to provide a hydrophobic surface. Silicone spacers (0.5 mm thick) were placed at the ends of the glass slide and a second hydrophobic-treated slide was placed on top; hence, the resulting uncured material had a uniform thickness of 0.5 mm to ensure uniform cure throughout the depth of the material. The slides were exposed to UV light (365 nm; IntelliRay 600W, Uvitron International, Inc.) of various intensities for various amounts of time to initiate crosslinking. Intensity was varied by placing the gels at a predetermined distance from the light source, where values

for light intensity as a function of distance were measured and provided by the manufacturer.

2.2.2. Free Radical-Polymerized Hydrogels. TEMED-cross-linked gels were prepared in a similar way as described in the previous subsection with several notable differences. Briefly, to prepare 5 ml of gel precursor solution, A, B, and deionized water were mixed at a desired ratio to give 4,972.5 μ l total volume. Upon degassing the precursor solution for 30 min, 25 μ l APS (10% w/v stock solution in deionized water; Bio-Rad, Hercules, CA) and 2.5 μ l of TEMED (Bio-Rad, Hercules, CA) were added. The solution was then again sandwiched between two hydrophobic-treated glass slides separated by spacers and left in a degassing chamber under vacuum for 45 min to polymerize.

2.3. Rheological Testing. Hydrogel slabs (0.5 mm thick) were swollen in PBS overnight and cut into circular slabs 20 mm in diameter for rheological testing. Residual buffer from the hydrogel surface was carefully blotted with a KimWipe[®] prior to measurement to avoid slipping. The storage modulus (G') and loss modulus (G'') were measured with an AR 2000ex rheometer (TA Instruments, New Castle, DE) in a parallel plate geometry with a 20 mm diameter, flat upper plate, at 22°C, a frequency of 1–10 rad/s, a constant 2% strain, and a normal force of 0.20–0.25 N [14]. Young's modulus was related to G' by the following equation:

$$E = G' 2(1 + \nu), \quad (1)$$

where E is Young's modulus and ν is Poisson's ratio which was approximated to 0.5 for PAA gels [15, 16].

2.4. Sample Preparation for Testing the Effect of Polymerization Conditions on Hydrogel Stiffness

2.4.1. Effect of UV Intensity. A8-B.10 and A12-B.25 hydrogel precursor solutions were prepared in 50 ml conical vials. To degas the hydrogel precursor solution, the conical vials were left uncapped and placed in a degassing chamber under vacuum for 30 min. Next, 350 μ L samples of each of the solutions were placed between two hydrophobic-treated glass slides. The slides were then placed in a UV oven with the following settings: exposure time of 300 s and/or 100 s and UV intensity of 15, 26, 67, and 131 mW/cm².

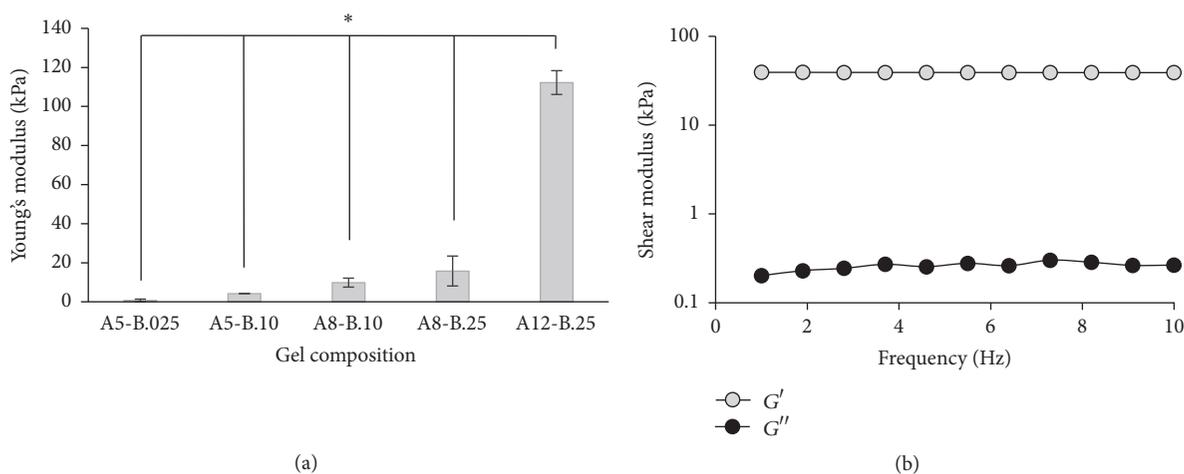


FIGURE 1: (a) Young's modulus as a function of gel composition for TEMED-crosslinked PAA hydrogels. (b) Representative shear stress as a function of frequency plot for A12-B0.25 PA hydrogel. Asterisks designate significant differences for $p < 0.05$, $n = 3$.

2.4.2. Effect of Exposure Time. A8-B.10 and A12-B.25 hydrogel precursor solutions were prepared in a 50 ml conical vial and degassed for 30 min, and 350 μL samples of each were placed between two hydrophobic-treated glass slides. The slides were then placed in a UV oven with the following settings: exposure time of 50 s, 75 s, 100 s, 125 s, and 300 s and UV intensity of 15 mW/cm^2 .

2.4.3. Effect of Irgacure Concentration. UV-crosslinked gels were prepared with various amounts of Irgacure 2959: 0.01%, 0.10%, and 0.50%. Precursor solutions of A8-B.10 gels were prepared, degassed for 30 min, and placed between two hydrophobic-treated glass slides. The slides were placed in the UV oven to polymerize at an intensity of 15 mW/cm^2 for 300 s.

2.4.4. Effect of UV Dose. PAA hydrogels were fabricated at different doses (i.e., total energy) calculated as follows:

$$\text{Dose} = \text{intensity} \times \text{time}, \quad (2)$$

where the dose is in mJ/cm^2 , intensity is in mW/cm^2 , and time is in s. UV-crosslinked gels were prepared at various doses ranging from $\sim 1 \text{ mJ}/\text{cm}^2$ to $\sim 40 \text{ mJ}/\text{cm}^2$. Two types of PAA gels were prepared for this experiment to compare the effects of dose on gels with different moduli: a stiff gel (A12-B.25) and a soft gel (A8-B.10). For the stiff gel, three different UV intensities were used (15, 26, and 67 mW/cm^2), while the exposure time was steadily increased to obtain increasing dose values. For the soft gel, the intensity was held at 15 mW/cm^2 , while the exposure time was again increased to obtain an increasing dose.

2.5. Collagen Coating of PAA Gels and Cell Culture. The PAA gels of desired moduli were prepared using UV-initiated polymerization. The PAA gels were coated with 0.1 mg/mL Type I collagen using a Sulfo-SANPAH crosslinker (50 mg/mL) as described by us previously [17]. Gel plates were then sterilized under UV light (302 nm) for 2 h. The coated gels

were then washed 2 times in PBS and equilibrated with 1x RPMI medium supplemented with 10% v/v FBS and 1% v/v pen/strep overnight. MDA-MB-231 human breast cancer cells were cultured on the hydrogels and maintained using regular cell culture techniques in RPMI medium supplemented with 10% v/v FBS and 1% v/v pen/strep. Cells were harvested through exposure to 0.5% v/v trypsin/EDTA for 5 min and seeded onto the PAA gels at a concentration of $1 \times 10^5 \text{ cells}/\text{cm}^2$. After supplementing with complete medium, cells were placed in a humidified incubator at 37°C and 5% CO_2 and cultured for 24 h.

2.6. Cell Imaging and Morphology. Cells were stained with acridine orange (green fluorescence, membrane permeable dye) at 24 h of culture on the PAA gels. Bright field and fluorescent images were captured using an inverted fluorescent microscope (Axiovert 200, Zeiss, Germany) with a 20x objective. Images were analyzed for cell spreading using the line plug-in on ImageJ (NIH free software).

2.7. Statistical Analysis. The results of all experiments are reported as the mean values ($\pm\text{SD}$) of triplicate samples performed in a minimum of three independent experiments. For measuring cell elongation, a total of ~ 75 –100 cells were analyzed from 9 images taken from three independent sample sets used for each condition. Comparisons between multiple groups were performed with single factor analysis of variance (ANOVA) followed by Tukey's post hoc test. Comparisons between two samples were performed with two-tailed Student's t -tests with post hoc analysis. Differences between two data sets were considered significant when $p < 0.05$.

3. Results and Discussion

While the use of TEMED-polymerized PAA hydrogels is common practice, UV-polymerized PAA gels for use as cell substrates are now gaining momentum. Although TEMED-polymerized gels offer a range of elastic moduli (Figure 1),

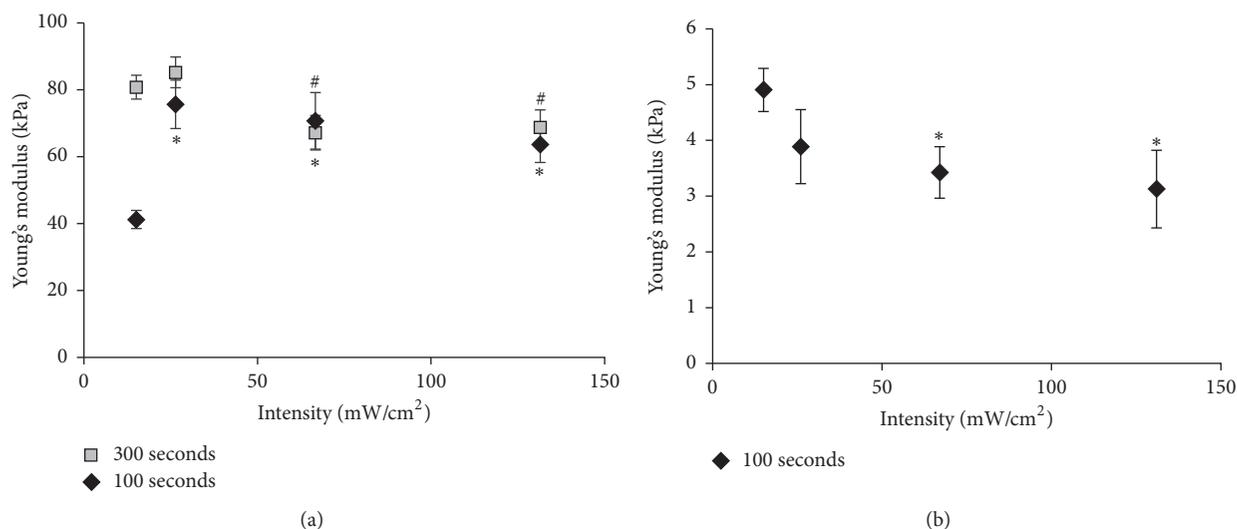


FIGURE 2: Young's modulus as a function of UV light intensity for (a) A12-B.25 and (b) A8-B.10, PAA hydrogels. Asterisks and pound symbols designate significant differences from Young's modulus for gels polymerized at 15 mW/cm² ($p < 0.05$, $n = 3$).

there are several distinct advantages to using UV polymerization. First, it allows for the formation of gradient gels which are excellent platforms for the investigation of cell motility, durotaxis, or differentiation [1, 10, 12]. Second, UV polymerization also allows for gel and surface patterning, providing a tool for evaluating cell behavior as a function of surface topography or composition [18, 19]. From a practical standpoint, UV crosslinking is faster; a typical gelation time for TEMED-based gels would be ~30–45 min, while the gelation time for UV-polymerized gels is ~1–5 min [1]. Lastly, the chemicals typically needed for UV polymerization (e.g., photoinitiators such as Irgacure) are less hazardous as opposed to the ones required for standard free radical polymerization, such as the toxic TEMED catalyst [8]. However, while both reactions are based on free radical polymerization, different parameters govern the polymerization kinetics and the resultant Young's modulus. Rather than trying to manipulate multiple parameters (UV exposure time and intensity) independently to obtain desired stiffness, we aimed to streamline the process, while simultaneously increasing reproducibility. Additionally, rather than using multiple gel compositions for subsequent experiments, we chose a soft hydrogel of lower acrylamide and bisacrylamide concentration (A8-B.10) and a stiff hydrogel of higher acrylamide and bisacrylamide concentration (A12-B.25). The A8-B.10 gel was chosen over the other soft hydrogels (A5-B.025 and A5-B.10), because it was easier to handle. Two different hydrogels were selected to ensure that the observed trends in modulus as a function of UV-polymerization parameters would hold for hydrogels of significantly different monomer and crosslinker concentration (i.e., resultant modulus).

3.1. Effect of UV Intensity. Here, we first examined the effect of light intensity on the final PAA hydrogel modulus (Figure 2). We specifically examined a soft (A8-B.10) and a

stiff (A12-B.25) PAA hydrogel at two different exposure times: 100 s and 300 s. We observed a bimodal dependence of gel modulus on UV light intensity. There was ~83% increase in gel stiffness with a 74% increase in light intensity (from 15 to 26 mW/cm²) for the stiff gel at a short exposure time of 100 s. However, rather than a further increase in modulus with an increase in intensity, we observed a slight, yet not statistically significant decrease in modulus. While not as significant, a similar trend was observed for the stiff gel at an exposure time of 300 s. There was a slight, but insignificant, increase of 6% in Young's modulus with an increase in UV intensity from 15 to 26 mW/cm², followed by a statistically significant 20% decrease in modulus for the higher UV intensities of 67 and 131 mW/cm². For the softer A8-B.10 gel at an exposure time of 300 s, the highest modulus was achieved at the lowest 15 mW/cm² UV light intensity; there was a 37% drop in modulus between gels made at 15 mW/cm² versus 131 mW/cm² intensity.

An increase in photopolymerized hydrogel modulus with increased light intensity was an anticipated result and can be explained by an improved crosslinking reaction and a greater number of functional crosslinks formed [20]. The second trend, which showed no further increase in modulus above a certain threshold UV light intensity, is not as well documented. For the free radical UV polymerization studied here, we postulate that the phenomena can be explained by overall faster polymerization kinetics and faster radical transfer, resulting in shorter polymer chains. Also, it is possible that complete photoinitiator consumption, leading to reaction completion past a threshold UV light intensity, can explain the lack of modulus increase. The rate of UV polymerization is directly proportional to the UV light intensity and photoinitiator concentration [21]. Thus, we note that, for a predetermined photoinitiator concentration, there was an optimal UV light intensity for achieving a higher modulus

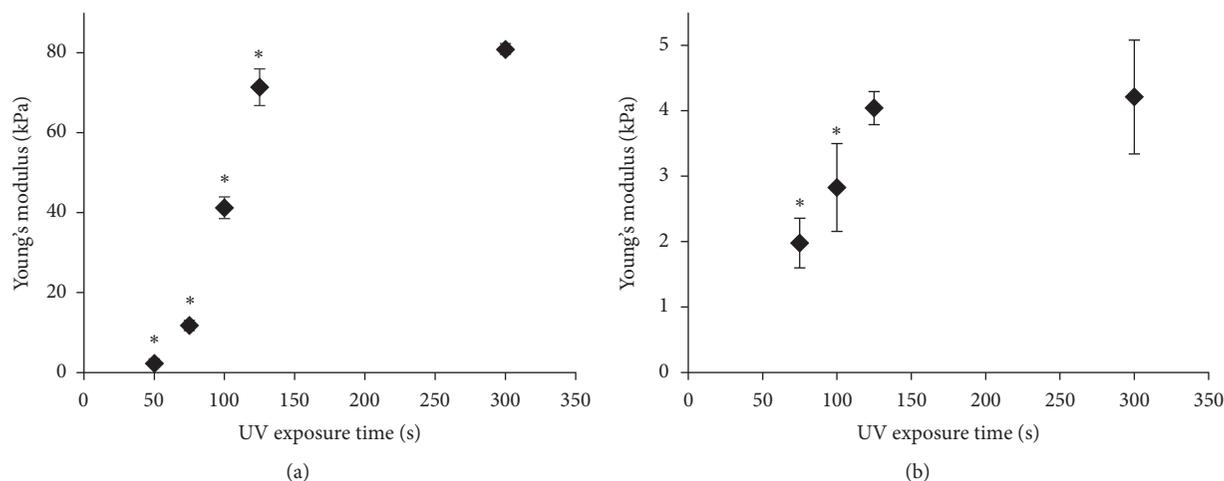


FIGURE 3: Young's modulus as a function of UV exposure time for (a) A12-B.25 and (b) A8-B.10, PAA hydrogels. Asterisks designate significant differences from Young's modulus for gels polymerized at an exposure time of 300 s ($p < 0.05$, $n = 3$).

from a given gel precursor solution, which was dependent on the total acrylamide and bisacrylamide concentration.

3.2. Effect of UV Exposure Time. For photopolymerized PAA hydrogels, UV exposure time has been utilized as the most effective strategy to achieve varying Young's moduli from the same hydrogel precursor solution [1, 10, 12]. As polymerization is a kinetic process, the longer exposure times allow for the reaction to proceed to completion. For this reason, longer polymerization times, up to a certain threshold, are expected to lead to a larger number of functional crosslinks, resulting in a higher Young's modulus. Further, it is possible that UV exposure time, by altering reaction rate, has an effect on the resultant hydrogel network defects, which have been shown to occur for PAA hydrogels, especially at higher acrylamide and bisacrylamide concentrations [22]. Increase in network defects (i.e., hydrogel heterogeneity) with an increase in reaction rate has been shown previously [21]. Network defects, on the other hand, have a pronounced effect on hydrogel modulus [23]. For example, chain entanglements cause an increase in crosslink density within the polymer network, which in turn results in a higher modulus [24, 25]. Alternatively, intramolecular cycles (chain loops) decrease crosslink density, thus negatively affecting hydrogel modulus [21]. Furthermore, since the polymerization rate depends on the concentrations of the acrylamide monomer and bisacrylamide crosslinker, they are expected to change for each precursor solution. Here, we again tested a soft gel (A8-B.10) and a stiff gel (A12-B.25). As expected, the polymerization rate was dependent on the total acrylamide and bisacrylamide concentration (Figure 3).

We noted that longer polymerization times were needed to reach the gelation point for precursor solutions of lower total acrylamide and bisacrylamide concentration. For example, while only a 50 s exposure time was required to form the stiff gel (Figure 3(a)), a minimum exposure time of 75 s was needed to achieve gelation for the soft gel (Figure 3(b)). However, for both gels, we noted a significant increase in

Young's modulus for higher exposure times. For example, for the A12-B.25 gel, we saw a 97% increase in Young's modulus for gels with an exposure time of 50 s versus gels with an exposure time of 300 s. For the A8-B.10 gel, we saw a similarly large increase of 53% for gels with an exposure time of 75 s versus gels with an exposure time of 300 s. Hence, by simply changing exposure time, we were able to achieve a large variation in stiffness in gels from the same gel precursor solution.

3.3. Effect of Photoinitiator Concentration on PAA Hydrogel Modulus. For UV-polymerized PAA gels, the photoinitiator Irgacure absorbs UV light to generate free radicals causing a chain reaction that leads to polymerization. The resulting polymer chain length affects the hydrogel modulus—the higher the chain length, the stiffer the resultant gel. One way to affect the polymer chain length is by altering the photoinitiator concentration. Increasing the photoinitiator concentration has been shown to result in multiple shorter polymer chains and hence a lower modulus [26]. Decreasing the photoinitiator concentration has been shown to result in a lower number, but higher molecular weight polymer chains leading to a higher modulus [26]. Our data corroborates previous findings: we observed a 1.3-fold increase in modulus when the Irgacure concentration was decreased from 0.5% to 0.1% and another 1.2-fold increase in modulus when the Irgacure concentration was decreased from 0.1% to 0.01% (Figure 4).

3.4. Effect of UV Dose. While above we demonstrated that both UV exposure time and UV light intensity could be used to control PAA hydrogel modulus, it is often impractical to test multiple samples to determine the optimal UV condition that would lead to an optimal hydrogel modulus. Along with manipulating intensity and time independently, we followed the evolution of hydrogel modulus as a function of total UV energy, that is, UV dose (see (1)). We again considered a soft gel (A8-B.10) and a stiff gel (A12-B.25). The data in Figure 5

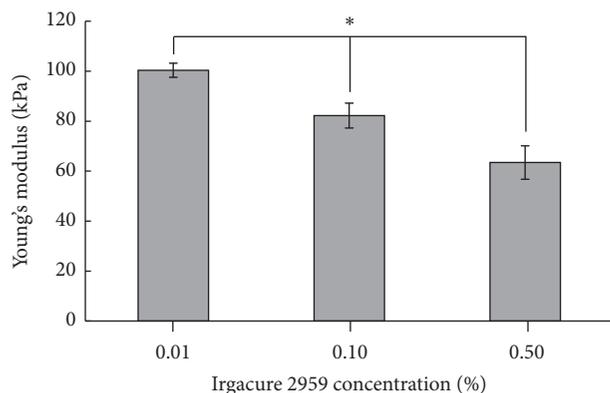


FIGURE 4: Young's modulus as a function of photoinitiator Irgacure 2959 concentration for A12-B.25 PAA hydrogel for a fixed UV intensity of 15 mW/cm² and fixed exposure time of 300 s. Asterisks designate significant differences for $p < 0.05$, $n = 3$.

represents the change in Young's modulus as a function of UV dose, where dose was varied by a change in UV exposure time for a fixed light intensity. When presented in this fashion, we saw an important trend emerge: there was a threshold dose of $\sim 5 \times 10^3$ mJ/cm² at which no further increase in Young's modulus was observed for any hydrogel tested. It is important to note that this threshold value was achieved by manipulating either the UV intensity or the UV exposure time and was independent of the total acrylamide and bisacrylamide concentrations tested in this study. However, it is conceivable that using a significantly lower acrylamide or bisacrylamide concentration would affect the threshold modulus; the concentrations here were chosen to simulate the modulus range of most soft tissues in the body [3]. To the best of our knowledge, this trend has not been previously reported for UV-polymerized PAA hydrogels.

Furthermore, we saw a steep increase in Young's modulus for minimal changes in dose in the range of 0.8×10^3 to 5×10^3 mJ/cm² and leveling off in the modulus for changes in dose in the range of 5×10^3 to 40×10^3 mJ/cm². Overall, the observed dose, as well as total time needed to fully polymerize the PAA hydrogel, aligned closely with data reported in the literature [12]. The above described trends along with the individual intensities and exposure times used are summarized in Table S1 (in the Supplementary Material available online at <https://doi.org/10.1155/2017/5147482>). Lastly, the moduli achieved via UV polymerization are similar to those measured when fabricating PAA hydrogels using traditional AP/TEMED polymerization (Figure 1). For example, for A12-B.25 UV-polymerized gel using UV intensity of 15 mW/cm² and 0.10% Irgacure concentration, Young's modulus was 93.8 ± 0.27 kPa, while the same gel made using AP/TEMED polymerization had Young's modulus of 112.3 ± 8.0 kPa. It is important to note that further optimization of UV-polymerization conditions to achieve a higher modulus is possible. For example, decreasing the Irgacure concentration ten times (from 0.1% to 0.01% w/v) increased Young's modulus of the A8-B.10 PAA and the A12-B.25 PAA gels, making them of the same modulus as the AP/TEMED-polymerized

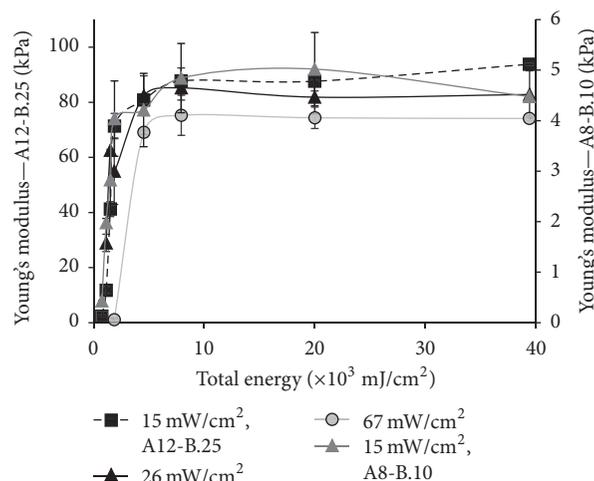


FIGURE 5: Young's modulus of two PAA hydrogel compositions as a function of UV dose calculated as UV intensity multiplied by UV exposure time.

hydrogels of the same composition (Table S2). Note that the exposure time was also increased for the lower Irgacure concentration to allow for complete polymerization to occur. This increase in modulus with the decrease in photoinitiator concentration could be attributed to higher molecular weight polymer chains (see Figure 4).

3.5. Cell Morphology on UV-Polymerized PAA Hydrogels. We also evaluated cell spreading on PAA hydrogels fabricated using UV polymerization. Our goal was to show that, regardless of the method with which the dose for PAA polymerization was obtained (i.e., high intensity but short exposure time versus low intensity by long exposure time), cell behavior would be identical on gels of identical stiffness. For this experiment, we maintained a constant dose of 8 mJ/cm² but altered the UV intensity and the exposure time. For one set of gels, the intensity was low (15 mW/cm²) and the exposure time was high (524 s). Consequently, for another set of gels, the intensity was high (67 mW/cm²) and the exposure time was low (119 s). We used tissue culture polystyrene (TCP) as control. We found that the cell spreading area on all three samples was $\sim 600 \mu\text{m}^2$ and that there were no significant differences (Figure 6). This result corroborates the cell spreading area observed on the TEMED-polymerized gels of similar stiffness (Figure 7), indicating that UV polymerization can be utilized to fabricate PAA hydrogels for the use of substrates to assess cellular behavior.

4. Conclusions

In conclusion, we present a comprehensive analysis on the effect of various crosslinking parameters on the final PAA hydrogels' Young's modulus. Manipulating PAA hydrogels modulus is becoming increasingly important in the fields of basic cell biology, biophysics, tissue engineering, and regenerative medicine, where PAA gels are widely used as cell culture substrates. Importantly, for UV-polymerized hydrogels, we

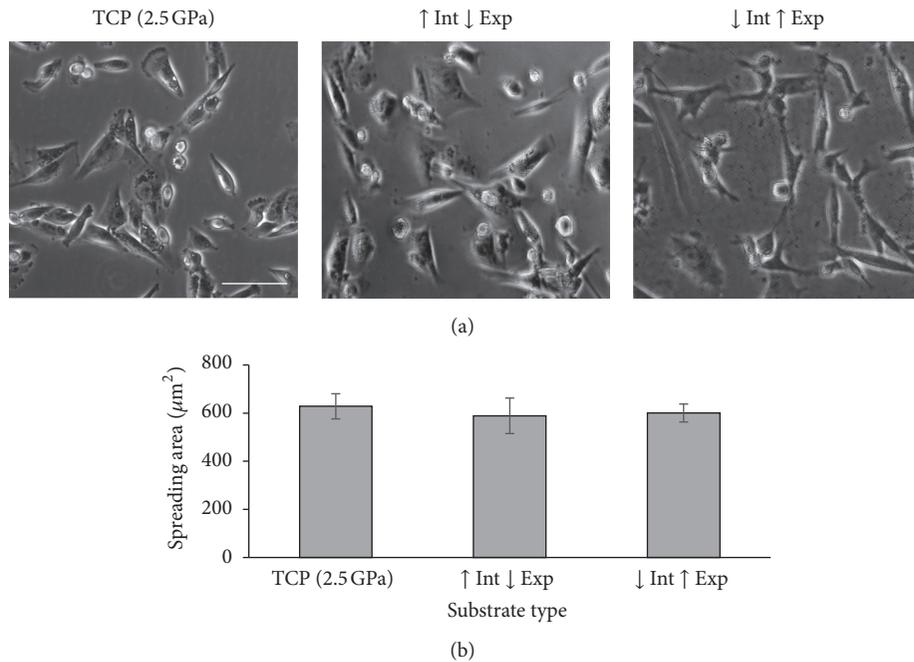


FIGURE 6: Cell morphology after 24 h of culture on collagen-coated UV-polymerized A12-B.25 PAA hydrogels. (a) Phase contrast images of MDA-MB-231 cells seeded onto PAA gels of equal dose, but with different exposure times and UV intensity. The “↑ Int ↓ Exp” condition had an intensity of $67 \text{ mW}/\text{cm}^2$ and exposure time of 119 s. The “↓ Int ↑ Exp” condition had an intensity of $15 \text{ mW}/\text{cm}^2$ and exposure time of 524 s. (b) No significant differences were found in the spreading area of MDA-MB-231 cells seeded onto PAA gels of equal dose ($8 \text{ mJ}/\text{cm}^2$), but with different exposure time and UV intensity (same as in (a)). Scale bar represents $100 \mu\text{m}$.

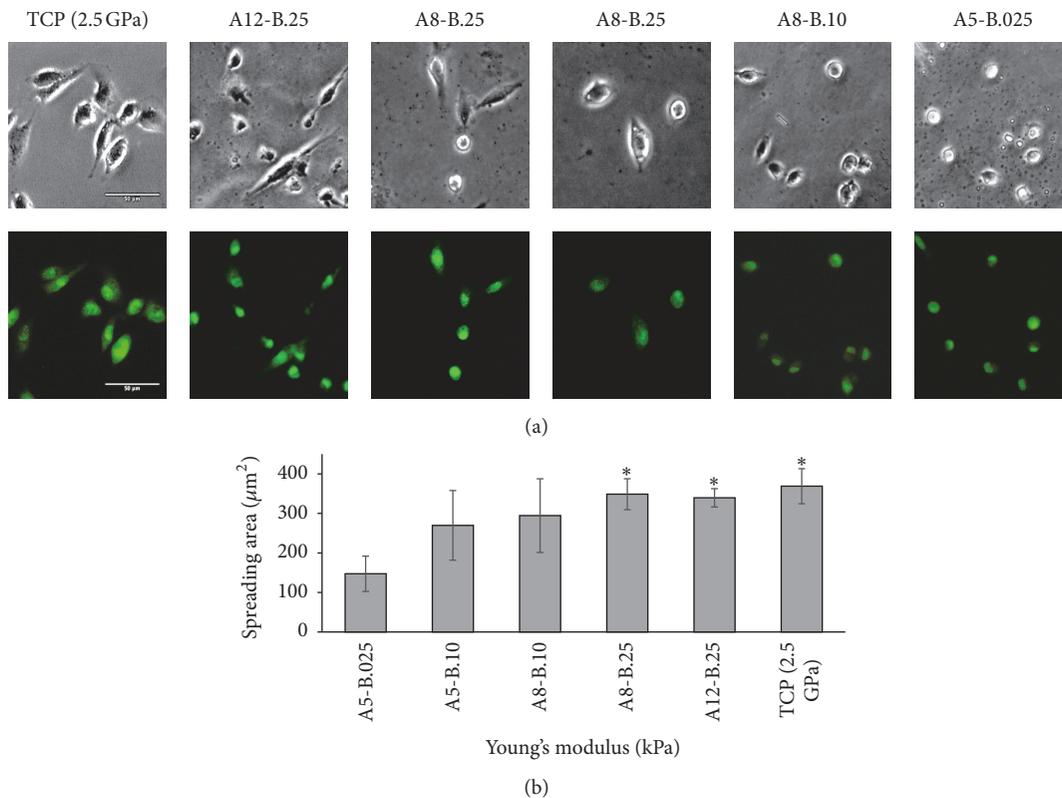


FIGURE 7: Cell morphology after 24 h of culture on collagen-coated PAA gels of varying stiffness. (a) Phase contrast (upper panel) and fluorescent (lower panel, acridine orange staining) images of MDA-MB-231 cells seeded on PAA gels of different stiffness. (b) Spreading area of MDA-MB-231 cells seeded on PAA gels of different stiffness. Asterisks designate significant differences from the 1 kPa hydrogel ($p < 0.05$, $n = 3$).

demonstrated that, rather than tuning the hydrogel modulus via UV intensity or exposure time independently, one should consider the UV dose for the system. For our system, which utilized 0.1% w/v of Irgacure photoinitiator, the threshold dose was found to be 5×10^3 mJ/cm²; below this energy, we saw an abrupt increase in compliance for incremental changes in energy, while we did not observe any significant changes in hydrogel compliance for any changes in UV dose above the threshold value. The cell spreading observed in PAA gels prepared using UV polymerization corroborated results seen in the TEMED-polymerized PAA gels. Our comprehensive analysis on the effect of implementing UV dose could serve as a useful guide to increase the reproducibility and ease of use for the fabrication of UV-polymerized PAA hydrogels for many research applications.

Disclosure

Hunter Stevenson's current affiliation is Department of Bioengineering, University of Texas at Dallas, 2850 Rutherford Ave., Richardson, TX 75080, USA. Era Jain's current affiliation is Department of Biomedical Engineering, Washington University in St. Louis, 6201 Forsyth Blvd., St. Louis, MO 63105, USA.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding this paper.

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