Cytocompatible cell encapsulation via hydrogel photopolymerization in microfluidic emulsion droplets

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Encapsulating cells within biocompatible materials is a widely pursued and promising element of tissue engineering and cell-based therapies. Recently, extensive interest in microfluidic-enabled cell encapsulation has emerged as a strategy to structure hydrogels and establish custom cellular microenvironments. In particular, it has been shown that the microfluidic-enabled photoencapsulation of cells within PEG diacrylate (PEGDA)-based microparticles can be performed cytocompatibly within gas-permeable, nitrogen-jacketed polydimethylsiloxane microfluidic devices, which mitigate the oxygen inhibition of radical chain growth photopolymerization. Compared to bulk polymerization, in which cells are suspended in a static hydrogel-forming solution during gelation, encapsulating cells via microfluidic processing exposes cells to a host of potentially deleterious stresses such as fluidic shear rate, transient oxygen depletion, elevated pressures, and UV exposure. In this work, we systematically examine the effects of these factors on the viability of cells that have been microfluidically photoencapsulated in PEGDA. It was found that the fluidic shear rate during microdroplet formation did not have a direct effect on cell viability, but the flow rate ratio of oil to aqueous solution would impart harmful effects to cells when a critical threshold was exceeded. The effects of UV exposure time and intensity on cells, however, are more complex, as they contribute unequally to the cumulative rate of peroxy radical generation, which is strongly correlated with cell viability. A reaction-diffusion model has been developed to calculate the cumulative peroxy radical concentration over a range of UV light intensity and radiation times, which was used to gain further quantitative understanding of experimental results. Conclusions drawn from this work provide a comprehensive guide to mitigate the physical and biochemical damage imparted to cells during microfluidic photoencapsulation and expands the potential for this technique. *Published by AIP Publishing.* [<http://dx.doi.org/10.1063/1.4993122>]

I. INTRODUCTION

Encapsulation within synthetic hydrogels is a promising and widely used approach to immobilize cells and protect them from mechanical stresses and deleterious macromolecules, including macrophages and antibodies, while allowing bidirectional diffusion of nutrients, oxygen, and wastes.^{1–4} Cell encapsulation strategies have been developed for tissue engineering and cell-based therapies, enabling the location of cells at injury sites or the continuous delivery of therapeutic reagents for chronic diseases. Cell encapsulation also suggests the potential for xenotransplantation⁵ and has been explored and employed as cell-based therapies for type 1 diabetes,⁶ vascular differentiation,⁷ and cartilage formation⁸ over the past few decades. Various natural polymer hydrogels have been explored for cell encapsulation including hyaluronic acid,⁹ agarose,⁶ dextran,⁷ and alginatepolykysine.¹⁰ Poly(ethylene glycol) (PEG)-based monomers

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have been widely employed and characterized for cell encapsulation due to their excellent biocompatibility, mechanical property, and tunable and readily modified network architecture.^{11–14} The functionalization of PEG with acrylate end groups to PEG diacrylate (PEGDA) enables the facile photopolymerization of PEG-based hydrogels and has dramatically expanded their role in cell encapsulation. The ease of PEGDA hydrogel-forming solution photopolymerization has subsequently enabled lithographic patterning, allowing precise temporal and spatial control over the hydrogel features.^{15,16}

More recently, microencapsulating cells within hydrogels ranging from 100 μm to 500 μm ¹⁷ has emerged as an area of intense research interest with broad utility in therapeutics,¹⁸ material synthesis,¹⁹ drug discovery,²⁰ and biosensing, in which the sensitivity and signal strength can be enhanced by up to 2 orders of magnitude compared to traditional methods due to the large surface to volume ratio.^{20,21} Similarly, the development of microfluidic technology for cell microencapsulation promises a dramatic enhancement to the high throughput handling of cells. High throughput cell encapsulation is based upon microfluidic emulsification in which highly monodisperse surfactant-stabilized aqueous microdroplets are formed at the junction of microfluidic channels or in coaxial capillaries.^{22–24} Microfluidic channel networks are commonly fabricated in polydimethylsiloxane (PDMS) using soft lithography, a fast and inexpensive fabrication process that generates devices via replica molding from a photolithographically patterned master.²⁵ PDMS molding has dramatically expanded the research activity and applications for microfluidic devices in biology and bioengineering due to the elastomer's cytocompatibility, high gas permeability, and rapid gas transport rates. While the permeability of air through PDMS is often a benefit, it can also present challenges during photopolymerization. Previous research has shown that the radical chain polymerization of PEGDA is strongly inhibited by the oxygen both in the system and in the air^{26,27} particularly when the experiment was conducted in the gas permeable PDMS-based microfluidic devices^{28,29} using the oxygen abundant fluorinated oil as the continuous phase.^{30,31} Oxygen inhibition particularly complicates cell encapsulation in microfluidic devices via peroxy radicals generated during the oxygen scavenging process,²⁷ which impart harmful effects to cells, including lipid peroxidation,³² amino acids oxidation,³³ inactivation of specific enzymes,^{34–36} and DNA damage.³⁷ Oxygen inhibition has been mitigated to photopolymerize PEGDA-based microparticles by fully polymerizing hydrogel-forming solution within emulsion drops within a recently described nitrogen-purged microfluidic device.³⁸ This nitrogen-purged device was further modified to significantly improve cell viability during photoencapsulation by enabling the precise control of oxygen in the emulsion by regulating the nitrogen purging pressure.³⁹

The photoencapsulation of cells within emulsion droplets produced by microfluidic devices is complemented by other recent developments in single cell handling. Inertial focusing, for instance, allows the precise alignment of cells to well-defined lateral and longitudinal locations without the need for external force.^{40–44} This high-throughput technology allows the single cell encapsulation in microdroplets⁴⁵ or microparticles for the analysis of cellular response⁴⁶ or products.⁴⁷ Despite the high flow rates required for inertial focusing, high post-processing cell viability has been reported and the technique has been repeatedly demonstrated to be gentle to cells.^{48,49}

During their microfluidic distribution into droplets and the subsequent photopolymerization of PEGDA hydrogel forming solutions within those droplets, cells are exposed to a host of potential deleterious factors. The cellular response to those individual factors, however, has not been systematically elucidated. In this work, we evaluate the effects of fluidic shear rate, cell residence time within microfluidic devices, nitrogen purging pressure, and UV exposure intensity and time on cell viability. While fluid flow conditions were found to dramatically affect cell viability, relative, not absolute, oil and aqueous flow rates were found to be the determining factor. To interpret the effects of UV exposure time and intensity on cell viability, a finite-element reaction-diffusion model was utilized to calculate the cumulative concentration of peroxy radicals for each condition. The results showed that exposure time and intensity, even at the same cumulative UV dose, contribute unequally to the aggregate production of peroxy radicals as determined by residual oxygen and its diffusion rate through the emulsion. By evaluating the cellular

response to those factors, this work offers clear guidance regarding device design and process conditions for optimized cell viability following microfluidic photoencapsulation.

II. EXPERIMENTAL SECTION

A. Cell culture

Human lung adenocarcinoma epithelial cells (A549) were routinely cultured using low-glucose Dulbecco's modified Eagles medium (DMEM; Sigma-Aldrich, USA) supplemented with 10% fetal bovine serum (Sigma-Aldrich, USA), 1% penicillin/streptomycin, and 0.2% Fungizone (Life Technologies, USA) at 37 °C and 5% CO₂ in a humidified incubator.

B. Microfluidic device fabrication

Master casts of the microfluidic device used in this work were fabricated using soft lithography techniques⁵⁰ according to previously described protocols.³⁹ For casts with only one layer of features, a silicon wafer (Silicon Inc. USA) was spin coated with an SU-8 2015 negative photoresist (MicroChem, MA, USA) to a thickness of 30 μm. A photomask with design features (CAD/Art Services, OR) was placed in contact with the photoresist film and exposed to collimated UV light (Omnicure S2000, USA) to achieve the polymerized features. The uncured photoresist was removed using a developer (Propylene glycol monomethyl ether acetate, Sigma-Aldrich, USA). A two-layer mold was photolithographically patterned for use as the nitrogen-purged microfluidic device. The first layer (30 μm) was first fabricated as described above, followed by the fabrication of a second planar flow network using SU-8 100 (MicroChem, MA, USA) at a thickness of 100 μm. Microfluidic devices were cast by pouring polydimethylsiloxane (PDMS) (Dow Corning, MI) upon the patterned silicon wafer, degassing, and curing at 70 °C for 4 hours. The cured elastomer replica was peeled from the mold and punched with a sharpened 20 G dispensing needle (Brico Medical Supplies, Inc., USA) to fashion inlet and outlet holes. PDMS replicas were bonded to glass slides after being exposed to oxygen plasma. Finally, Aquapel (PPG Industries) was briefly injected into the device and flushed with nitrogen to obtain a hydrophobic surface for aqueous droplet generation.

C. Macromolecule synthesis and hydrogel photopolymerization

A549 cells were cultured to 80%–90% confluence according to previously described^{38,39} protocols. Confluent cells were trypsinized using 0.05% trypsin/ethylenediaminetetraacetic (Life technologies, USA), pelleted, and resuspended in heavy phosphate-buffered saline (PBS, Sigma-Aldrich, USA) ($\sim 3 \times 10^7$ cells/ml), which was adjusted with OptiPrep density medium (Sigma-Aldrich, USA) to have a specific density of 1.05 g/ml. A 45 total wt. % macromer mixture was prepared in PBS consisting of 75 mol. % polyethylene glycol monoacrylate (PEGMA, Mn $\approx$ 400 Da; Monomer-Polymer and Dajac Laboratories) and 25 mol. % poly(ethylene glycol) diacrylate (PEGDA, Mn $\approx$ 3400 Da; Jenkem Technology, Beijing, P.R. China).⁵¹ The UV initiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) was synthesized as previously described^{52,53} and dissolved in PBS to a concentration of 0.3 wt. %. The cells in heavy PBS, UV initiator, and macromer solution were separately injected into the microfluidic device, in which they were merged and fully mixed to form the hydrogel-forming polymer solution consisting of 1×10^7 cells/ml, 0.1 wt. % LAP, and 15 total wt. % macromer.

D. Cell viability assessment

Cells encapsulated in microdroplets or microgels were separated from oil and washed on a 5-μm cell strainer (pluriSelect, USA) using PBS with 0.1 wt. % Pluronic F-127 and resuspended in PBS. Cell viability was determined using a LIVE/DEAD Viability/Cytotoxicity Kit for mammalian cells (Life Technologies, USA), in which staining solutions (2 μM calcein AM and 4 μM ethidium homodimer) were added and incubated for 15 min at room temperature. Stained hydrogels were washed with PBS, the number of live and dead cells were counted using an

inverted fluorescence microscope IX-71 (Olympus, USA), and the corresponding cell viability was calculated.

E. Fluidic shear rate and residence time

Cells and hydrogel-forming macromer solution were mixed within the device and then merged with the continuous phase, Novec 7500 containing 2 wt. % Pico-Surf (Dolomite, USA), to form droplets at the constriction of a microfluidic channel junction [Fig. 1(a)]. The cellular response to the fluidic shear rate was evaluated by regulating the absolute flow rates, as well the ratio between the dispersed phase and the continuous phase. Encapsulated cells were recovered and assayed to assess viability. While maintaining a constant fluid velocity (and therefore shear rate) and nitrogen-purging pressure ($P_{N_2} = 7$ psi), the residence time of cells in microfluidic devices was regulated by changing the length of the outlet channel. In this fashion, the cellular response to residence time within the device and nitrogen purge pressure was evaluated.

F. Nitrogen-purge pressure

The cellular response to nitrogen-purge pressure was evaluated by varying the purge pressure and the corresponding cell viability. When the nitrogen pressure was less than a threshold point, fully polymerized PEGDA-based microparticles could not be attained, indicating a basal background oxygen flux sufficient to compensate for oxygen consumption during photopolymerization. In cases in which no polymerization occurred, cells were recovered directly from the oil using the previously described particle recovery protocol, and cell viability was assayed.

G. UV radiation intensity and time

The effect of the total UV radiation exposure dose on cell viability was decoupled into exposure time and exposure intensity and was evaluated separately. A mask with a transparent region was placed upon the microfluidic device to define the UV exposure time (exposure time being equal to the length of the transparent region divided by the droplet velocity), and the UV exposure intensity was regulated using the Omnicure S2000's internal attenuation. The effect of UV exposure time on cell viability was evaluated by varying the exposure time from 1.1 s to 9.9 s with a constant UV intensity of 120 mW/cm². The cellular response to UV intensity was assessed by assaying cell viability at discrete UV intensity values ranging from 60 to 600 mW/cm² with a constant UV exposure time of 1.1 s.

H. Photopolymerization modeling

A reaction-diffusion model of free radical photopolymerization within microfluidic emulsion droplets³⁹ was used to quantify peroxy radical accumulation and interpret the effect of UV exposure on cell viability. A finite element numerical model was created using COMSOL multiphysics software using physical parameters and settings that are reported in the [supplementary material](#). A line drawn in 1-D axisymmetric space was used to represent a distance from the droplet center to the droplet interface. The time-dependent transport of diluted species study was selected across the line as the model basis using the following differential equations, which were solved simultaneously:⁵⁴

$$R_I = -k_d[I],$$

$$k_d = \varphi \varepsilon(\lambda, I) I \left(\frac{\lambda}{N_A h c} \right),$$

$$R_M = -k_p[X][M],$$

$$R_X = k_d[I] - k_t[X]^2 - k_{O_2}[X][O_2],$$

$$R_{O_2} = -k_{O_2}[X][O_2],$$

$$R_P = k_t[X]^2,$$

$$R_{ROO} = k_{O_2}[X][O_2].$$

In this droplet photopolymerization model, the main reacting species were macromer, photoinitiator, oxygen, and free radicals, where X represents all the radical species (M^* and R^*) as described in [supplementary material Table II](#). All reaction parameters used in this model were obtained from the literature.^{52,55–59} Based upon experimental observations of nitrogen jacket effectiveness, an assumption was made that the oxygen concentration in a droplet within a purged microchannel was sufficiently dilute. The pressure-dependent oxygen concentration, 0.01 mol/m^3 , was calculated using data from this experiment, which was used as the basis for solutions via the reaction-diffusion model.³⁸

III. RESULTS AND DISCUSSION

A. Cellular response to fluidic shear rate and residence time

Cells flowing within the hydrogel-forming macromer solution were pinched by the continuous phase, Novec 7500 containing 2 wt. % Pico-surf, to form microdroplets at the nozzle of the microfluidic device [Fig. 1(a)]. The fluid flow rate has been previously shown to affect the behavior and function of bacteria⁶⁰ and cells.⁴⁸ Also fluid velocity has been related to the cell rotation rate in flowing fluids⁶¹ and the stress force imparted to cells⁶² and was postulated to

FIG. 1. Effect of fluidic flow on cell viability: (a) Schematic of the microfluidic device used to assess the cellular response to the fluidic shear rate. (b) Cell response to changes in flow rates with a constant ratio (2:1) between the oil and aqueous phases. (c) Cell response to changes increasing flow rate ratio between the oil phase and the aqueous phase at a constant aqueous flow rate of $2 \mu\text{L}/\text{min}$. (d) Deformation of cells via extensional flow during droplet generation. The black dotted line indicates the position at which the aqueous and oil streams merge. The progression of droplet formation (left to right) accelerates the cell the final droplet velocity. (e) Cell velocity as a function of its downstream position after entering the droplet junction and outlet channel, with a starting position of zero indicated by the dotted line in (d). (f) Cell viability as a function of maximum cell acceleration during droplet generation. (g) Elongation of cells as they pass through the droplet generating nozzle.

cause physical damage to cells. The flow rates of the oil phase and aqueous phase were varied by either maintaining a constant flow rate ratio between the two or maintaining a constant aqueous flow rate, $2\ \mu\text{l}/\text{min}$ and increasing the oil flow rate. Droplets were collected and cells were separated from the oil phase as described. The effect of the fluidic shear rate on cell viability was mapped [Figs. 1(b) and 1(c)], in which the cell viability was unchanged while the flow rate was increased from 3 to $39\ \mu\text{l}/\text{min}$ with a constant ratio, 2:1, between the oil phase and the aqueous phase. However, cell viability dropped from $>95\%$ to 65% as the flow rate ratio between the oil phase and the aqueous phase increased from 2:1 to 15:1. This disconnect can be explained by the local velocity change of cells during the droplet generation process, in which cells were accelerated to the velocity of the oil phase as they were encapsulated into droplets. Cell velocity was mapped in relation to their position relative to the nozzle and cell acceleration was determined. Figure 1(d) shows the path followed by a cell as it is encapsulated within a discrete droplet. The dashed line indicates the merging of the aqueous and oil channels and all positions are referenced to this line. Acceleration is reported as a change in local fluid velocity as a function of linear position along the channel. The acceleration of the cell across the flow focusing junction is not a function of the absolute flow rates but instead the relative ratio of aqueous:oil flow rates. As anticipated, this result illustrates that the acceleration of cells increased as the ratio of oil phase to aqueous phase flow rates increased. To examine the effect of this elongational force on cells during droplet generation, cell viability was evaluated as a function of acceleration [Fig. 1(f)], in which smaller acceleration did not have an effect on cell viability. Upon reaching a threshold, however, acceleration became increasingly harmful to cells, which is consistent with the relationship between cell viability and flow rate ratio of an oil-to-aqueous emulsion forming solution. The observed deformation of cells during encapsulation is a hallmark of increased oil:aqueous flow rate ratios and Fig. 1(g) characterizes the relationship between cell deformation and its acceleration [Fig. 1(e)] as they pass through the nozzle with a specific oil:aqueous flow rate ratio (30:2). In this work, it has been shown that high linear velocities through the device, and during encapsulation did not have harmful effects on cells; however, high oil to aqueous flow rate ratios do cause physical damage to cells by elongating them, thus leading to a dramatic decrease in post-encapsulation viability.

This result reaffirms a host of previous reports that describe the cytocompatible encapsulation and retention of cells within microfluidic droplets. A unique and unexplored feature of the current photoencapsulation platform, however, is the passage of cells within droplets through a nitrogen purged, oxygen depleted channel network. The residence time of cells through the device was regulated by varying the outlet positions on the device [Fig. 4(a)], and the travel time of cells in the device was calculated using the total flow rate of the fluids and geometry of the microfluidic device. The cellular response to in-device residence time under a high nitrogen purging pressure ($P_{\text{N}_2} = 7\ \text{psi}$), as required for viable cell encapsulation,³⁹ was evaluated as shown in Fig. 2. The influence of residence time over the range of required exposure times (2.4 s to 14.4 s) was shown to be minimal, as cell viability did not change significantly. These empirical observations indicate that neither residence time nor the high nitrogen-purging pressure is by themselves deleterious to cells. Therefore, microfluidic device operating conditions that provide precise control of oxygen concentration via nitrogen-purging required to achieve encapsulating cells in fully polymerized PEGDA microgel particles also permit high cell viability.

B. Cellular response to nitrogen purging during photopolymerization

Nitrogen purged microfluidic devices have been successfully demonstrated to mitigate oxygen inhibition of free chain polymerization, achieve fully polymerized microparticles³⁸ via PEGDA photopolymerization, and enable viable cell encapsulation.³⁹ To evaluate the influence of nitrogen-purge pressure on cell viability during photopolymerization, nitrogen pressure was increased from 0 to 7 psi. Flow rates of the hydrogel-forming solution and oil phase were $1\ \mu\text{l}/\text{min}$ and $2\ \mu\text{l}/\text{min}$ for the first layer and $7\ \mu\text{l}/\text{min}$ for the second layer, respectively, corresponding to an exposure time of 0.35 s. Microdroplets containing hydrogel forming solution and cells were exposed to $220\ \text{mW}/\text{cm}^2$ UV light while traversing the UV exposure region. The

FIG. 2. The effect of residence time of droplet-encapsulated cells traveling through a device on cell viability, in which the total flow rate was $3 \mu\text{l}/\text{min}$ and the nitrogen purging pressure is 7 psi. Droplets were not photopolymerized in this work to isolate the effects of encapsulation and in-droplet residence.

results illustrate a clear relationship between cell viability and nitrogen purge pressure. Figure 3 shows that cell viability increased from 20% to 90% as the nitrogen pressure was ramped from 0 to 7 psi. Cell viability has been previously been related to the aggregate generation of peroxy radicals during radical oxidation.³⁹ These observations confirm that the nitrogen purge pressure directly and quantifiably influences the oxygen concentration in the emulsion and indicate a minimum viable purging pressure to avoid cell damage.

C. Cellular response to UV exposure dose: Time and intensity

Having established a target nitrogen purging pressure to mitigate the deleterious effects of oxygen byproducts during continuous photoencapsulation, the influence of UV exposure dose was assessed by independently varying exposure time and UV intensity. While nitrogen purging has been shown to enable high viability cell encapsulation within fully polymerized PEGDA-based microparticles, the effects of UV radiation on cell viability have not been clearly illustrated. Our proposed reaction-diffusion model predicts polymerization of the entire microdroplet once oxygen has been depleted in both the aqueous and oil phases. This assumes

FIG. 3. Effect of nitrogen purging pressure during photopolymerization on cell viability, where microfluidic droplets were exposed to $220 \text{ mW}/\text{cm}^2$ UV light for 0.35 s. Nearly full viability was recovered at a purge pressure of 7 psi, indicating a strong and linear relationship between nitrogen pressure and residual oxygen in the PDMS.

that nitrogen purging either eliminates or reduces the flux of oxygen through the PDMS to significantly less than the rate of consumption during photopolymerization. Therefore, it follows that the kinetics of photopolymerization will strongly dictate the overall accumulation of peroxy radicals. Here, we decoupled the total UV radiation dose used to achieve full polymerization by independently varying UV exposure time and intensity. Their effect on cell viability was evaluated separately.

To control the UV exposure time, a series of transparent masks with discrete illumination lengths were designed and placed on the top of the microfluidic device as shown in Fig. 4(a). Throughout this time excursion, the UV intensity was maintained at a constant 220 mW/cm². The effect of UV exposure time on cell viability is presented in Fig. 4(b). Cell viability dropped from 90% to 20% while the UV exposure time was increased from 1.1 s to 9.9 s. These results indicate that the consumption of oxygen within the droplet and subsequent photopolymerization of the hydrogel forming PEGDA solution occur quickly at the given UV intensity. An increase in exposure time merely allows additional oxygen to diffuse through the oil phase and into the particle, where it is converted to reactive oxygen species (ROS). The effect of UV intensity on cell viability was next evaluated by varying the UV intensity while maintaining a constant UV exposure time, 1.1 s. The cells' response to varied UV intensity was mapped as shown in Fig. 4(c), in which cell viability was not noticeably affected by UV intensity over the range of 60 to 600 mW/cm². Collectively, these data conclusively inform polymerization conditions within the device: high UV intensities should be utilized to photopolymerize PEGDA droplets. High UV intensity over short exposure times minimizes the conversion of oxygen to ROS. When lower UV intensities are used over longer exposure times, full particle polymerization can be achieved but at slower rates. Full polymerization is achieved only after all residual oxygen is consumed in both the droplet and oil phases. The conversion of additional oxygen to ROS scales with exposure time and leads to a proportional decrease in cell viability. It is therefore clear that cell viability is not proportional to the overall UV exposure dosage (mJ) but the accumulated conversion of oxygen during the photopolymerization process.

D. Calculated cumulative peroxy radical generation

To illustrate the relative effects of UV exposure time and intensity on oxygen conversion, we highlight two sets of experimental conditions in which the total UV radiation doses were equal. At UV radiation times of 5.5 s and 1.1 s and UV intensities of 120 and 600 mW/cm², respectively, the UV exposure doses were equal, yet the cell viabilities diverged: 60% and 85%, respectively. To quantify why the effects of the same UV radiation dosage were different on cell viability, the cumulative peroxy radical amount formed during the UV exposure using our reaction-diffusion COMSOL model. This model generated quantitative estimates of ROS generation (Fig. 5) and highlights the divergence in viability between longer UV exposure times and higher UV intensity. Experimental results, analyzed in the framework of a reaction-

FIG. 4. (a) Schematic of the device used to regulate the UV exposure time by controlling the size of the transparent area on the mask. (b) The effect of UV exposure time on cell viability was mapped, in which the UV intensity was held at a constant 220 mW/cm². (c) Effect of UV intensity on cell viability was evaluated, in which the UV radiation time was fixed 1.1 s and the intensity increased.

FIG. 5. Solid circles represent the cumulative concentration of reactive oxygen species (ROS) as a function of UV exposure time with a constant UV intensity of 220 mW/cm². Solid triangles represent the cumulative concentration of peroxy radicals as a function of UV intensity under the identical UV exposure time of 1.1 s.

diffusion oxygen balance, illustrate that longer UV exposure times are considerably more deleterious to cells than high UV intensity.

IV. CONCLUSIONS

In this study, we have fully evaluated cellular responses to individual factors involved in the microencapsulation of cells via photopolymerization of PEGDA within microfluidic emulsion droplets. Residence time, nitrogen purge pressure, and absolute fluid flow rates were all analyzed and found to minimally impact cell viability, even at high values. During photopolymerization, however, cell viability was extremely sensitive to nitrogen purge pressure. The nitrogen purge pressure should be, at minimum, 5 psi in order to achieve a high post-encapsulation cell viability. Cellular response to UV exposure illustrated that the UV exposure time and intensity contribute disproportionately to the formation of peroxy radicals during the cell encapsulation. Perhaps counterintuitively, short, high intensity exposure conditions should be adopted to minimize the accumulation of generated peroxy radicals. This integrated analysis of cell response to these individual factors illustrates that, within normal operating parameters, cell viability during microfluidic photoencapsulation is most sensitive to the aggregate generation of ROS. Taken together, these results provide a complete framework for the design and operation of microfluidic devices for the high throughput photoencapsulation of cells within hydrogel microparticles. More broadly, these results may also inform the heterogeneity of cell response during cell encapsulation within bulk hydrogels.

SUPPLEMENTARY MATERIAL

See [supplementary material](#) for a summary of oil and cell suspension flow rates, which correspond to data in Figs. 1(b), 1(c), and 1(f). [Supplementary material](#) Table II summarizes the PEGDA polymerization reaction mechanism, and Table III lists physical parameters and settings used to create the reaction-diffusion model used to generate in Fig. 5. Supplementary Fig. 1 illustrates cellular acceleration across a flow focusing junction at high flow rates.

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