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Comparative cytocompatibility of multiple candidate cell types to photoencapsulation in PEGNB/PEGDA macroscale or microscale hydrogels

Zhongliang Jiang, Kun Jiang, Ralph McBride, and John S. Oakey

Department of Chemical Engineering, University of Wyoming, Laramie, USA

Abstract

The encapsulation of live cells into photopolymerized hydrogel scaffolds has the potential to augment or repair tissue defects, establish versatile regenerative medicine strategies, and be developed as well-defined, yet tunable microenvironments to study fundamental cellular behavior. However, hydrogel fabrication limitations constrain most studies to macroscale hydrogel scaffolds encapsulating millions of cells. These macroscale materials possess regions of heterogeneous photopolymerization conditions and are therefore poor platforms to identify the response of individual cells to encapsulation. Recently, microfluidic droplet-based hydrogel miniaturization and cell encapsulation offers high-throughput, reproducible, and continuous fabrication. Reports of post-encapsulation cell viability, however, vary widely among specific techniques. Furthermore, different cell types often exhibit different level of tolerance to photoencapsulation-induced toxicity. Accordingly, we evaluate the cellular tolerance of various encapsulation techniques and photopolymerization parameters for four mammalian cell types, with potential applications in tissue regeneration, using polyethylene glycol diacrylate (PEGDA) or polyethylene glycol norbornene (PEGNB) hydrogels on micro- and macro- length scales. We found PEGNB provides excellent cellular tolerance and supports long-term cell survival by mitigating the deleterious effects of acrylate photopolymerization, which are exacerbated at diminishing volumes. PEGNB, therefore, is an excellent candidate for hydrogel miniaturization. PEGNB hydrogel properties, however, were found to have variable effects on encapsulating different cell candidates. This study could provide guidance for cell encapsulation practices in tissue engineering and regenerative medicine research.

Keywords

cell microencapsulation; cytocompatibility; droplet-microfluidics; cell dependent tolerance

Introduction

Current demand for tissue or organ repair, augmentation, or even transplantation have been increasing worldwide, and far outpacing therapeutic availability[1]. Cell-based therapies are a promising strategy for direct tissue repair or regeneration[2], and cell candidates have been favorably selected, intensively studied, and used to validate materials, principles, and practices for new tissue engineering and regenerative medicine concepts. For instance, mesenchymal stem cells (MSCs), powerful multipotent progenitor cells, have been

extensively studied due to their capacity for differentiation and secretion of many stimulating trophic factors[3]. Fibroblasts of mesenchymal origin can migrate and proliferate in response to chemotactic stimuli and are valued for their potential to remodel extracellular matrix (ECM) by regulating epidermal proliferation and differentiation[4]. Increasingly, tissue engineering strategies are being applied to developing an understanding of tumor tissue and cancer progression, utilizing a variety of cell lines of tumorigenic origin[5,6]. And, finally, natural and synthetic tissue scaffolds have been used in an attempt to recapitulate lost organ function. To address Type 1 diabetes, efforts have been increasingly focused on understanding and engineering regulatory pathways that control the production and secretion of insulin, on both molecular and cellular levels. For such cell-delivery-based regenerative medicine practices, there is an urgent unmet need to design biocompatible, selectively porous barriers for the delivery and implantation of exogenous cells[7]. A unifying challenge to these diverse applications is designing and providing customized growth environments, which includes guiding cues associated with environment, development stage, and extracellular communication[8,9]. Synthetic hydrogels excel at this task and have been copiously studied as cell scaffolds, most commonly on macroscopic, or bulk length scales, upon which hydrogels are formed within molds possessing dimensions on the order of millimeters to centimeters, therefore, lacking control over cell distribution or network homogeneity.

Cell encapsulation within hydrogel scaffolds offers a range of powerful and versatile strategies for tissue engineering[10] and regenerative medicine[11]. Hydrogels possess high water content[12] and permit bidirectional diffusion of nutrients and metabolic waste[13], thereby permitting cellular viability and functionality post encapsulation. Semi-permeable hydrogel networks can also protect cells by preventing the immune system from detecting and terminating exogenous targets[14,15]. Additionally, cell encapsulation within hydrogel matrices modified with biological ligands provides a 3D platform that emulates the *in vivo* environment, allowing the elucidation of cellular mechanisms in a well-defined, tunable environment[16,17]. Previous attempts have demonstrated the bulk encapsulation of stem cells[18], fibroblasts[19], and pancreatic β -cells[20,21] into hydrogel scaffolds for tissue engineering, repair, and regenerative medicine, respectively. Chondrogenesis of stem cells[22], migration and activation of fibroblasts[23], and survival and cytokine secretion of pancreatic β -cells[24] have been successfully achieved via dynamic control over hydrogel properties, along with understanding of fundamental cell-cell or cell-matrix interactions. Although bulk cell encapsulation and subsequent implantation has shown promising clinical outcomes[25,26], bulk gels are limited by relatively low diffusivity[27] and a lack of control over individual cell behavior and response to encapsulation, which can result in wide and unpredictable experimental variability. Moreover, the screening and identification of improved matrix formulations is hindered by low experimental throughput and analysis in bulk gels. The clinical and translational potential for bulk gels is also limited by the need to surgically implant large, cellularized hydrogels. Accordingly, *in situ* forming injectable hydrogels have been widely studied[28,29].

The miniaturization of bulk hydrogel scaffolds into microscale injectable cell carriers has also been more recently demonstrated in combination with a variety of approaches to overcome design constraints inherent to bulk hydrogels[30,31]. These efforts, including

liquid bridging[32], stop flow lithography[33], and bioprinting [34,35], have successfully reduced the physical size of individual hydrogels, and therefore decreased diffusion lengths. By coupling these fabrication methods with custom materials chemistry, the functionality of the microgels may be engineered, as with programmed degradation[33,36], directed microgel assembly[37], or controlled cell interactions[38,39] for *in vitro* studies. The production and collection of microgels by these techniques, however, is considerably constrained by the fabrication approach, which dramatically hinders their translational potential. To increase injectable microgel fabrication throughput, while retaining precise control over microgel size and shape, microfluidic-based droplet forming techniques have introduced the capability to produce monodisperse cell-laden hydrogel-forming droplets at kHz rates[40-45]. Combined with inertial focusing for precise control over intervals between cells[46-48], microfluidic droplet platforms have enabled high throughput single cell encapsulation and subsequent molecular analysis, such as screening and sorting[49-51]. These techniques provide new high throughput methods to explore the heterogeneity of encapsulated cell populations, and thus understand the complex regulatory pathways contributing to the functionality of tissues[52,53]. Post-encapsulation cell viability has been considered as a critical factor allowing for either *in vitro* cell studies, or *in vivo* functional tests. Previous attempts to encapsulate cells into microgels have produced high initial viability, however a dramatic decrease in viability is typically seen over longer time periods[54,55]. Previous studies have considered encapsulation procedures and materials chemistry independently and have determined that microfluidic handling and encapsulation are cell friendly, thus identifying materials chemistry as the primary factor determining postencapsulation viability. As such, polymer and hydrogel chemistry must be further investigated to understand its role in optimizing live cell encapsulation, supporting long-term high cell viability, and providing a salubrious environment for cell growth and tissue elaboration.

Pioneering work has demonstrated cell microencapsulation using both natural and synthetic materials[56-59]. Although natural materials exhibit excellent biocompatibility, their lack of chemical and mechanical tunability and insufficient control over gelation kinetics make natural materials difficult to micropattern and inadequate to satisfy the broad range of properties demanded of a versatile cell encapsulant. Additionally, issues associated with immunogenicity, protein purification and pathogen transmission have tremendously constrained the clinical potentials of natural materials[60]. Synthetic polyethylene glycol (PEG)-based hydrogels have been intensively studied and employed as a cell encapsulant[61], primarily due to their bioinert nature[62], high biocompatibility[63], and the ease with which chemical properties may be tuned[64]. The biocompatibility of PEG hydrogels enables the *in vivo* implantation and injection of encapsulated cells, while mitigating immunorejection[65]. In recent years, microgels have been fabricated using a chemically-initiated Michael-type addition crosslinking scheme[66], which showed excellent functionality and biocompatibility[67]. However, this scheme requires the reaction between a nucleophilic thiolate and an electrophile, which initiate a rather rapid chemical reaction to form a thioester linkage[68], so miniaturized hydrogel features are cumbersome to produce[67], and its application in microfluidic devices is inherently limited. PEG-based free radical initiated photopolymerization has been developed, where photolabile o-nitro

benzyl-containing hydrogels have enabled spatial and temporal control of gelation and hydrogel mechanical properties[69-72] and *in situ* polymerization[73]. Two crosslinking mechanisms are commonly utilized to achieve gelation, including crosslinking between polyacrylates formed by chain-growth (PEGDA), or thiol-ene linkages (PEGNB) formed by step-growth polymerization[74]. PEGDA has been widely explored for its potential as a cell carrier and scaffold material over the past few decades[27,75-78]. It has been well established that PEGDA polymerization is inhibited by oxygen-induced free radical scavengers[79] that hinder full gelation of PEGDA hydrogels, which produces a negligible effect in macroscale gels but becomes increasingly problematic at cellular length scales. More notably, reactive oxygen species (ROS) generated as byproducts are deleterious to cells[80,81], dramatically reducing post-encapsulation viability. Studies have shown that hyaluronic acid attenuates ROS levels in cartilage and, accordingly, hyaluronic acid has been incorporated into PEG-based cell encapsulants[82-84]. In most recent years, Anseth and coworkers have demonstrated PEGNB as a novel cell encapsulant, which not only maintains all the versatile aspects of PEGDA, but also has superior polymerization kinetics and well-organized network formation[85-87]. High long-term cell viability has been achieved in PEGNB microgels[88], however, different cell types consistently display variable response to encapsulation within hydrogels of varying macromolecular structure, composition, and physical properties[89,90].

Challenges to hydrogel miniaturization and single cell encapsulation result from issues that were not anticipated by results with bulk polymerized gels and were not appreciated until recently. For instance, the continuous polymerization of cytocompatible PEGDA formulations in microfluidically formed droplets has been frustrated by oxygen inhibition[91], until the incorporation of nitrogen-purging channels within polydimethylsiloxane (PDMS) devices[92]. The accumulation of ROS during polymerization likely hinders short and long-term cell survival by inducing apoptosis[55]. Conversely, these complications are not observed with photopolymerized PEGNB[88], possibly due to the fact that the polymerization of PEGNB can consume ROS[93]. The residence time of cells in hydrogel forming solution, however, is a crucial parameter that impacts long-term cell viability[88]. Flow conditions and the use of certain photoinitiators[94] can also potentially damage cells. These issues are either compounded or mitigated by different polymeric hydrogelforming materials, thus establishing unique conditions for applications in microfluidics. As an example, our previously reported PEGNB microgel platform demonstrates significantly different behavior relative to PEGDA microgels[88]. In order to develop an empirical understanding of the impact of different factors on cell viability, and more importantly, to develop a cell microencapsulation platform that can be universally tolerated by most cell types, we have explored the cytocompatibility of four mammalian cell candidates under different photoencapsulation conditions. In this study, we first compared the toxicity of initiator concentration, UV irradiation intensity and duration, on PEGDA or PEGNB cell encapsulation to establish photopolymerization parameters with minimized toxicity. Changes in surface to volume ratio of the encapsulating hydrogel phase, considered to be a key differentiating factor between macroscale and microscale hydrogel encapsulation, was analyzed to better understand cell viability in diminishing volumes. Next, we circumvented the limitations of both materials in

microfluidics by introducing a nitrogen-jacketed microfluidic device to polymerize PEGDA microgels on device, and batch polymerization to polymerize PEGNB microgel aliquots in a centrifuge tube. Finally, we compared viability results in macroscale hydrogels as a standard against which to validate the biocompatibility and feasibility of our microencapsulation platforms. We have shown that our PEGNB-based microencapsulation platform is highly biocompatible with most of the selected mammalian cell candidates, particularly in comparison to a similarly designed PEGDA platform. PEGNB hydrogel properties, however, can affect cell viability to varying degrees. Therefore, hydrogel properties must be matched to specific cell candidates in order to establish viable cytocompatible cell microencapsulation platforms. The high long-term survival rate of these cells, selected for their utility across a range of tissue engineering and regenerative medicine applications, would facilitate downstream functionality studies of the encapsulated cells. Additionally, the tight and controllable size distribution achievable with microfluidic encapsulation, along with up to 1kHz production rate of PEGNB microgels, provides insight into high throughput single cell encapsulation and analysis. Overall, this study provides guidance for general cell encapsulation practices that can be broadly applied to tissue engineering and cell-based therapeutic strategies.

Materials and Methods

Macromer and initiator synthesis

The macromer species, PEGNB, was synthesized in consultation with established procedures as described elsewhere[80,88,95,96]. Briefly, 10 g of 4-arm PEG ($M_n \approx 20k$, JenKem Technology) was placed in a 250 mL round bottom flask containing 20 mL of $MeCl_2$ and set aside to dissolve at room temperature while stirring. Into a separate 50 mL round bottom flask containing a stir bar was added 0.54 g of N,N-dicyclohexylcarbodiimide (DCC, Sigma Aldrich, USA) followed by dissolution in 10 mL of $MeCl_2$. To this solution 0.70 g of 5-norbornen-2-carboxylic acid (5N2B, Sigma Aldrich) was added dropwise. The contents were placed under Argon and allowed to stir for 30 minutes at room temperature whereupon the initially clear solution turned cloudy white. To the 250 mL round bottom flask containing fully dissolved PEGNB was added 0.21 g of pyridine and 0.032 g of 4-dimethylamino pyridine (both Sigma Aldrich, USA). The 250 mL round bottom flask was fitted with a medium-mesh fritted glass filter (Ace glass) and vacuum adapter and set in an ice bath. The contents of the 50 mL round bottom flask were filtered via vacuum into the 250 mL flask, stirred, and allowed to react under Argon for 24 hours. Product-containing solution was washed twice with 5% $NaHCO_3$, once with brine, then precipitated in ice cold diethyl ether. Precipitate was filtered on a Butner funnel, then placed in a Soxhlet extractor fitted with an Allihn condenser and washed with gently boiling ether for 48 hours. The product was removed and dried under vacuum for 24 hours. The degree of functionalization (>90%) was confirmed via 400 MHz proton NMR using d_2 -DMSO as solvent[93].

The initiator species was synthesized in accordance with previously published procedures [88,94,97]. Briefly, 3.0 g of 2,4,6-trimethylbenzoyl chloride (Sigma Aldrich, USA) was added dropwise to a 250 mL round bottom flask containing an equimolar amount of dimethyl phenylphosphonite and stirred at room temperature under nitrogen overnight. A

four-fold excess of LiBr (Sigma Aldrich, USA) dissolved in 100 mL of methyl ethyl ketone (MEK, Sigma Aldrich, USA) was added to the flask and heated to 50 °C for 10 minutes. Upon cooling to room temperature overnight white crystalline salts were formed. Product crystals were filtered on a Buchner funnel and rinsed with ice cold MEK, then placed under vacuum until constant weight was achieved. Product was confirmed via 400 MHz proton nuclear magnetic resonance (NMR).

Microfluidic device fabrication

Microfluidic devices were designed and fabricated as previously reported[55,88]. Conventional soft lithography technique was used to fabricate the devices[98]. Briefly, PDMS was poured onto a silicon wafer (Silicon Inc, USA) with designed micropatterns, vacuumed for 30 minutes to remove the entrapped air, then transferred to a 70 °C oven overnight to cure. PDMS replicas were then trimmed and punched with a 20 G dispensing needle (CML Supply, USA) to fashion inlets and outlets. After cleaning with ethanol, PDMS replicas were bond to glass slides after exposing to oxygen plasma (Harrick Scientific, USA), then transferred to 70 °C oven to facilitate bonding. Channel dimensions ($h \times w$) of double layer nitrogen jacketed device for polymerizing PEGDA microgels were $30 \mu\text{m} \times 30 \mu\text{m}$ for the first layer, and $100 \mu\text{m} \times 100 \mu\text{m}$ for the second layer. To improve feasibility and reproducibility enabled by polymerization kinetics of PEGNB, a simplified cross-flow microfluidic device was designed. Channel dimensions ($h \times w$) were $80 \mu\text{m} \times 100 \mu\text{m}$ for cell-containing hydrogel forming solution, and $80 \mu\text{m} \times 30 \mu\text{m}$ for the oil phase.

Hydrogel forming solution

PEGNB hydrogel forming solutions were mixed with dithiol linker, LAP, and cell-containing culture media to a final concentration of 10 wt% PEGNB, 10 mM dithiol linker ($M_n \approx 500$ Da, Sigma Aldrich, USA), 0.1 wt% LAP for preparing 10 wt% PEGNB hydrogels[99]. To vary macromer concentrations, 20 wt% PEGNB, 20 mM dithiol linker, 0.1 wt% LAP were mixed for preparing 20 wt% PEGNB hydrogels, and 30 wt% PEGNB, 30 mM dithiol linker, 0.1 wt% LAP were mixed for preparing 30 wt% PEGNB hydrogels. A final concentration of 0.05 wt % thiolated Rhodamine B was added to label the PEGNB microgel network.

PEGDA hydrogel forming solutions were mixed to a final concentration of 10 wt% PEGDA ($M_n \approx 3400$ Da, JenKem Technology) and 0.1 wt% LAP. To vary PEGDA concentrations, 20 wt% and 30 wt% PEGDA were mixed with 0.1 wt% LAP for polymerization. A final concentration of 0.01 wt% acrylate Rhodamine B was added to label the PEGDA microgel network.

The average molecular weight of each PEG base was approximately conserved between PEGNB and PEGDA used in this work, which have been shown to have similar mechanical properties and mesh size[100,101].

Microgel fabrication on device

To facilitate droplet generation, all of the microfluidic devices were filled with Aquapel (PPG Industries) and flushed with nitrogen to establish hydrophobic channel surfaces before

experiments. To fabricate 10 wt% PEGNB microgels, hydrogel forming solution was injected into a flow focusing microfluidic device via syringe pumps (NEMESYS, Cetoni GmbH, Germany). Flow rate of aqueous phase was held constant at 2 $\mu\text{L}/\text{min}$, while flow rate of fluorocarbon oil (Novec 7500 with 2 wt% Pico-Surf (Dolomite) as surfactant) was varied from 10 $\mu\text{L}/\text{min}$ to 50 $\mu\text{L}/\text{min}$ to control the size of droplets. Subsequently, these droplets were collected into a 1.5 mL microcentrifuge tube (VWR, USA), and then exposed to UV light (100mW/cm², Lumen Dynamics Group Inc, S2000-XLA, Canada) for 20 seconds to achieve gelation.

For 10 wt% PEGDA microgels, flow rates were kept constant at 1 $\mu\text{L}/\text{min}$ for aqueous phase, 2 $\mu\text{L}/\text{min}$ and 6 $\mu\text{L}/\text{min}$ for the first and second layer of oil phase. Nitrogen purging pressure was held constant at 7 psi. Droplets were polymerized by exposure to UV light (Mecury-100W, Chiu Technical Corporation, USA) through a 20X objective lens (Olympus) while passing through a serpentine channel in the second layer of the device.

PEGNB or PEGDA microgels were separated from oil by washing and centrifuging on a 10 μm cell strainer (PluriSelect, Germany) with 0.1 wt% Pluronic F-68 (Sigma-Aldrich, USA) in phosphate buffered saline (PBS, Life Technologies, USA).

Rhodamine B with thiol or acrylate ends (NANOCs, US) was copolymerized to label the network of PEGNB or PEGDA, respectively[88]. Microgels were imaged using fluorescence microscopy with a Texas Red filter cube, and their diameters were measured in ImageJ. 50 microgels were counted for each group.

Cell culture and harvest

Human lung adenocarcinoma epithelial cells (A549s) were cultured at 37 °C and 5% CO₂ in Dulbecco's Modified Eagle's Medium (DMEM, Life Technologies, USA) with low glucose supplemented with 10% fetal bovine serum (FBS, Life Technologies, USA), 1% Antibiotic-Antimycotic (Life Technologies, USA). Culture media was changed every 3 days and cell populations were subcultured every 6 days.

3T3 fibroblasts were cultured at 37 °C and 5% CO₂ in Dulbecco's Modified Eagle's Medium (DMEM, Life Technologies, USA) with low glucose supplemented with 10% fetal bovine serum (FBS, Life Technologies, USA), 1% Antibiotic-Antimycotic (Life Technologies, USA). Cell populations were subcultured every 3 days.

Equine mesenchymal stem cells (MSCs) were kindly received from Dr. John Kisiday's lab at CSU, Fort Collins, USA. MSCs were cultured at 37 °C and 5% CO₂ in Dulbecco's Modified Eagle's Medium (DMEM, Life Technologies, USA) with low glucose supplemented with 10% fetal bovine serum (FBS, Life Technologies, USA), 2ng/mL FGF-2 (Sigma- Aldrich, USA), 1% Antibiotic-Antimycotic (Life Technologies, USA). Culture media was changed every 3 days and cell populations were subcultured every 6 days. Since MSCs are primary cells, so cells before passage 4 were used for experiments.

Beta-TC-6 cells were obtained from American Type Culture Collection (ATCC, CRL-11506, Virginia, USA). The cells were cultured at 37 °C and 5% CO₂ in Dulbecco's Modified Eagle's Medium (DMEM, Life Technologies, USA) with high glucose supplemented with

15% heat-inactivated fetal bovine serum (FBS, Life Technologies, USA), 1% Antibiotic-Antimycotic (Life Technologies, USA). Culture media was changed every 3 days and cell populations were subcultured every 6 days.

To prepare cells for encapsulation, cells were detached from culture flasks with TrypLE Express (Life Technologies, USA), pelleted, and resuspended to a final concentration of 5×10^6 cells/mL in culture media.

UV irradiation intensity and time on cell viability

A549s were mixed with 10 wt% PEGNB or 10 wt% PEGDA hydrogel forming solutions with 0.1 wt% LAP into 2 mL microcentrifuge tube caps (VWR, USA). The 60 μ L mixtures were then exposed to UV light (Lumen Dynamics Group Inc, S2000-XLA, Canada) with varying intensities ranging from 10 mW/cm² to 600 mW/cm² while keeping exposure time constant as 20 seconds, or varying exposure time from 2 seconds to 120 seconds while keeping UV intensity constant at 100 mW/cm². Note that total UV irradiation dose received per hydrogel construct for each parallel experiment is the same.

LAP concentration on cell viability

A549s were mixed with 10 wt% PEGNB or 10 wt% PEGDA hydrogel forming solutions with 0.1 wt%, 0.5 wt% or 1 wt% LAP into 2 mL microcentrifuge tube caps, then the mixtures were exposed to UV light at 100 mW/cm² for 20 seconds for PEGNB or 5 seconds for PEGDA.

Hydrogel surface area effect on cell viability

A549s were mixed within 10 wt% PEGNB or 10 wt% PEGDA hydrogel forming solutions with 0.1 wt% LAP, then 60 μ L cell-laden hydrogel forming solutions were transferred onto either 2 mL microcentrifuge tube caps to make hydrogel discs with thicknesses of approximately 1 mm, or between two glass cover slips with the top slide coated with a uniformly thin layer of PDMS to make hydrogel thin films with thicknesses of $\sim 185 \mu$ m. Discs and films were photopolymerized as described above. The hydrogel surface area in contact with an oxygen source, and thus the surface area providing continuous oxygen diffusion during polymerization, was calculated. Hydrogel discs had a total surface area approximately 80 mm², with a diameter of 8.7 mm. Thin films were pressed into squares measuring 18 mm on a side, to give a total surface area of 324 mm².

Bulk cell encapsulation

Four cell types were mixed within PEGNB or PEGDA hydrogel forming solutions with variable macromer concentrations and exposed to UV light at 100 mW/cm² for 20 seconds for PEGNB or 5 seconds for PEGDA to achieve gelation. The approximate volume for each hydrogel construct is 60 μ L and the density of cells in the hydrogel was held constant at 5×10^6 cells/mL. The cell-laden hydrogel constructs were then cultured in incubator to monitor the longterm cell viability.

Microfluidic cell encapsulation and collection

Cells were suspended at a concentration of 5×10^6 cells/mL in culture media, and then mixed within PEGNB or PEGDA hydrogel forming solutions. To fabricate 10 wt% cell-laden PEGNB microgels, cells were well-mixed in hydrogel forming solutions by vortex, and aqueous and oil phase flow rates were held constant at 2 $\mu\text{L}/\text{min}$ and 10 $\mu\text{L}/\text{min}$, respectively. Due to the increased viscosity when increasing macromer concentration, in order to achieve stable droplet formation while keeping aqueous phase at a constant flow rate, flow rates for oil phase were held at 20 $\mu\text{L}/\text{min}$ for 20 wt% and 30 $\mu\text{L}/\text{min}$ for 30 wt% PEGNB hydrogel forming solutions. These flow rate conditions proved to be cell-friendly. Cell-laden droplets were then exposed to UV light (100mW/cm², Lumen Dynamics Group Inc, S2000-XLA, Canada) for 20 seconds for proper polymerization.

For PEGDA cell encapsulation, cells, macromer, and LAP were loaded into three syringes and mixed within microfluidic device with a serpentine channel. Flow rates were kept constant at 0.33 $\mu\text{L}/\text{min}$ for each of the aqueous phase, 2 $\mu\text{L}/\text{min}$ and 6 $\mu\text{L}/\text{min}$ for the first and second layer of oil phase. Nitrogen purging pressure was held constant at 7 psi to create a low oxygen environment. Droplets were polymerized by exposure to UV light through a 20X objective lens when passing through a serpentine channel.

PEGNB or PEGDA microgels were separated from oil by centrifuging on a 10 μm cell strainer as discussed above. Cell-laden microgels were resuspended into culture media containing 0.1 wt% Pluronic F-68 in ultra-low attachment 6-well plates (Corning Incorporated, USA), then transferred into an incubator for long-term culture with media change every other day.

Cell viability assay and measurement

Viability of encapsulated cells was measured by staining with Live/Dead viability kit (Life Technologies, USA), a cellular membrane integrity assay that stains live cells with green fluorescence and dead cells with red fluorescence. Cell viability was imaged using an inverted fluorescence microscope (IX-71, Olympus, USA) and calculated from three images.

Results and Discussion

Size distribution of microgels fabricated by microfluidic devices

PEGNB or PEGDA microgels were fabricated by using two different microfluidic device designs (Fig. 1). Thiolated or acrylated Rhodamine B was added into hydrogel forming solution to co-polymerize with PEGDA to form a fluorescently labeled hydrogel network. Microgels were therefore able to be imaged via fluorescence microscopy. PEGDA microgels fabricated microfluidically possessed a diameter of approximately 50 μm . Exploiting the facile control over fluid flow rates and velocity provided by microfluidics allows the precise and dynamic control over droplet sizes, allowing PEGNB microgels to be fabricated with diameters ranging from 50 μm to 160 μm with high monodispersity (Fig. 2(a) and (b)). It has been reported that encapsulating cells, especially cell clusters, using microfluidic devices has proven challenging due to the clogging of cells in microchannels[67]. Additionally, and perhaps most critically, photopolymerization of PEGDA droplets is susceptible to oxygen

inhibition, requiring purging to allow gelation and inhibit the production of ROS. These two considerations dramatically hinder the feasibility of microfluidic processing and reduce cell viability. To enable cell microencapsulation within more biocompatible, photopolymerizable materials, we have simplified the design of PEGNB encapsulation scheme by using a cross-junction device with larger dimensions. Flow rates of aqueous and oil phase were held at 2 $\mu\text{L}/\text{min}$ and 10 $\mu\text{L}/\text{min}$, respectively, to produce larger droplets for cell encapsulation (Fig. 2(c)). The diameter of PEGNB microgels over all flow rate ratios is below 200 μm , which is ideal for gas and nutrient exchange to and from encapsulated cells[54].

UV irradiation intensity, time and LAP concentration on cell viability

Bulk polymerization was conducted by polymerizing hydrogel constructs within caps of microcentrifuge tube to verify the toxicity of photopolymerization parameters on cells, and thus optimize cell survival after encapsulation, which can establish baselines for cell microencapsulation. UV irradiation intensity and time were varied independently while keeping the total UV dose delivered to each hydrogel construct constant (Fig. 3(a)). By increasing UV intensity from 10 mW/cm^2 to 600 mW/cm^2 at a constant irradiation time of 20 seconds, resulting in total UV dosage of 200 mW/cm^2 , 400 mW/cm^2 , 1000 mW/cm^2 , 2000 mW/cm^2 , 4000 mW/cm^2 , 6000 mW/cm^2 , and 12000 mW/cm^2 per hydrogel construct, no significant difference in viability was observed between cells following their encapsulation in PEGNB or PEGDA constructs. More than 90% of cells remained viable after staining with 2 μM calcein AM and 4 μM ethidium homodimer-1 (Fig. 3(b)), indicating cells can tolerate high transient UV intensity levels. When UV intensity was held at 100 mW/cm^2 and UV irradiation time was varied from 2 seconds to 120 seconds, however, cells in PEGDA hydrogels suffered from a dramatic decrease in viability while those in PEGNB hydrogel maintained much higher viability levels (Fig. 3(c)). As the same concentration of LAP and total dose UV was used for polymerizing PEGDA hydrogels in each parallel experiment, the difference in cell viability is solely attributable to the elevated cumulative production of ROS. As previously shown[102], the rate of ROS production is governed by UV exposure time, and is independent of UV intensity. Increased exposure time allows consumed oxygen to be replenished by diffusion into hydrogels, where it inhibits initiator and monomer radicals and is quickly converted into ROS[102]. However, cells in PEGNB hydrogels can tolerate high UV intensity as well as long UV exposure time, indicating the deleterious effect of ROS must be mitigated during PEGNB polymerization[87]. These results demonstrate that cellular tolerance is proportional to cumulative ROS, as opposed to total UV exposure dose and that, unlike PEGDA, PEGNB hydrogel encapsulated cells are tolerant of a broad range of UV exposure parameters.

To achieve sufficient gelation[103,104] while maintaining high initial cell viability, UV intensity was held constant at 100 mW/cm^2 for both PEGNB and PEGDA hydrogels, with PEGNB hydrogels exposed for 20 seconds, and PEGDA hydrogels for 5 seconds due to their variable polymerization kinetics [74,105,106]. 0.1, 0.5, and 1 wt% of the photoinitiator, LAP, was mixed into hydrogel forming solutions for polymerization. As expected, cells could not tolerate high LAP concentration in both hydrogel systems because of the elevation of ROS level (Fig. 3(d)). Cells lost their viability in a LAP concentration dependent manner, where LAP itself has been validated as cytocompatible[55]. Although cell viability in

PEGNB remained slightly higher than that of PEGDA as LAP concentration was increased, this difference was reduced relative to the lowest LAP concentration. Previous studies have postulated that ROS can be consumed by PEGNB hydrogels, explaining the higher observed post-encapsulation cell viability relative to comparable PEGDA hydrogel-forming formulations [80,88,107]. If this hypothesis is valid, our observation indicates that there is a threshold concentration above which the effect is saturated and ROS-induced cell damage cannot be further mitigated. Accordingly, for our further experiments, 0.1 wt% LAP was used to minimize toxic effects on cells while maintaining sufficient gelation.

Hydrogel surface area on cell viability—The overall ROS burden to which encapsulated cells are exposed depends upon the amount of oxygen initially present, plus that which diffuses into the hydrogel forming solution during photopolymerization. Therefore, hydrogel surface area, which quantitatively determines oxygen flux from the ambient environment, should correlate with the production of ROS during hydrogel polymerization. Thus, cell viability decrease in PEGDA should be proportional to surface area. To validate this hypothesis, we fabricated PEGNB or PEGDA hydrogel thin films, using equivalent volumes of hydrogel forming solution as in the hydrogel discs prepared previously. As such, thin films possessed a larger surface area to volume ratio by a factor of 4.05. Viability of A549s in PEGDA thin films was 10% lower than that in PEGDA hydrogel discs (Fig. 4), indicating hydrogel surface area is a defining factor in altering cell viability. However, no decrease in cell viability was observed between hydrogel discs and hydrogel thin films made from PEGNB, which can be correlated again to the hypothesis that polymerization of PEGNB can either inhibit the production of ROS or consume ROS before they attack cells. This experimental result is significant for understanding cell-encapsulation in microscale particles, as well as recursively understanding shape and dimensionality effects in bulk PEGDA gels[33], thin films[7], PDMS micromolding[108], and interfacial polymerization[109].

Cell-dependent tolerance in macro- and micro-scale encapsulation systems—As demonstrated by cell encapsulation in macroscale hydrogels, minimization of ROS is the key to high viability. Understanding this process can be applied to designing salubrious cytoencapsulation processes on the microscale, as well. In order to develop a fundamental understanding of cell-dependent tolerance to photoencapsulation into hydrogels, A549s, 3T3 fibroblasts, MSCs, and β -cells were encapsulated into PEGNB or PEGDA bulk hydrogel discs with varying macromer weight fractions to alter gel elasticity and mechanical strength[75], which provide mechanical support to encapsulated cells and affect cellular responses including migration[110], differentiation[111], and activation[112,113]. Polymerization conditions and protocols were replicated as described above for bulk hydrogels. A549s, 3T3 fibroblasts and MSCs responded positively to encapsulation within 10 wt% PEGNB hydrogels.

While almost 100% of cells survived immediately following encapsulation in 10 wt% PEGNB hydrogels, viability decreased slowly over the course of 14 days. At the end of two weeks, more than 80% of encapsulated A549s and 3T3 fibroblasts, along with 70% of MSCs remained viable. At an increased macromer concentration of 20 wt%, 3T3 fibroblasts can

maintain their viability at a relative high level comparable to the 10 wt% PEGNB condition, while A549s and MSCs typically started to die off after a week of culture, despite high initial cell viability observed. When macromer concentration was increased to 30 wt%, 3T3 fibroblast viability once again remained high (60%) relative to A549s and MSCs, which dramatically lost viability over the course of 14 days. For cells encapsulated into PEGDA hydrogels, even though initial cell viability can be maintained at a relative high level in 10 wt% PEGDA hydrogels, a dramatic decrease was observed in stiffer PEGDA hydrogels after culturing for 24 hours (Fig. 5(a), (b) and (c)). Notably, β -cells are the only cell candidate that displayed a negative response to photoencapsulation into both materials under all the conditions (Fig. 5(d)). Due to the fact that β -cells are highly sensitive to ROS[80], they were expected to show a significantly lower viability in PEGDA hydrogels. On the other hand, β -cells are also known to be highly cellular interactive and communication between cells is considered crucial for their survival[115]. Thus, hydrogel network formation may isolate β -cells, constraining their cellular interactions and resulting in decreased viability. The preassembly of single β -cells into islets before encapsulation[115], increasing β -cell density[114], functionalizing scaffolds with short peptides[21], or the use of cell-degradable materials[95] may elucidate factors governing encapsulated β -cell viability.

To recapitulate cellular responses from bulk hydrogel encapsulation, and also to explore the versatility and cytocompatibility of our microfluidic-based cell microencapsulation platform, four cell candidates were encapsulated into PEGNB or PEGDA microgel particles using a previously described microfluidic emulsification technique[55,88]. The viability of four cell types encapsulated into PEGNB microgels with varying macromer weight fractions showed very similar trends to that in PEGNB bulk hydrogels, indicating the excellent cellular tolerance of PEGNB bulk hydrogels can be recapitulated by PEGNB microgels fabricated via microfluidics. However, all cell types suffered from a massive loss in viability 24 hours after encapsulation and polymerization into 10 wt% PEGDA microgels, indicating cells may have initiated apoptosis upon polymerization due to the intense oxidative stress posted on them during ROS conversion (Fig. 6). Stiffening PEGDA microgels only made viability decrease more dramatically (data not shown) as expected based upon bulk results and previous literature reports.

To summarize, cells universally prefer low weight fraction hydrogels in either bulk or micro encapsulation schemes. 3T3 fibroblasts, as an exception, can maintain a high viability in stiffer PEGNB hydrogels. This can be likely explained by the nature of this specific cell type, which prefers to migrate and proliferate on stiff substrates. Additionally, during in vivo disease progression, ECM stiffness is an environmental cue for fibroblast recruitment, activation and remodeling of the local network[110,112]. The macromer concentration-dependent decrease in long-term cell viability indicates that 10 wt% PEGNB photoencapsulation displays minimized toxicity and can be tolerated by most cell candidates. Ideally, when macromer composition and molecular weight is fixed for step-growth polymerizations, crosslinking density and equilibrium swelling of a hydrogel should be independent of macromer concentration[93]. Practically, however, when low macromer concentrations were used, network defects can occur, which affect structural ideality[96]. Therefore, macromer concentration-dependent swelling and mechanical properties have been experimentally observed, which was also reported for chain-growth hydrogels[116].

The increase in macromer concentration can improve hydrogel mechanical strength[113], however, sufficient transport of nutrient and gas is strongly inhibited by the corresponding decrease in mesh size, presumably contributing to this significant loss in long-term cell viability[59,75]. Regardless of hydrogel network properties, cells can tolerate PEGNB better than PEGDA with the variable cell tolerance potentially resulting from their polymerization kinetics, in which the crosslinking of PEGNB is organized – in contrast with the random crosslinking of PEGDA – which results in more stable biophysical network formation[117]. Most importantly, previous work has indicated the network forming process of PEGNB may consume ROS, which can directly mitigate the deleterious effects of produced ROS[87]. Additionally, studies have shown that step growth thiol-ene reactions require quantitatively fewer radicals than that for acrylate reactions, and also the radicals in thiol-ene systems have shorter lifetimes[80,107]. Moreover, the activity of functional proteins markedly decreased in acrylate-based hydrogels, while remaining unchanged in thiol-ene systems[107].

Variable cell tolerance to cell type, scaffold material, and encapsulation

strategy—Over two weeks in culture, all tested cell types exhibited decreased viability as expected, but to different degrees. More than 50% of A549s, fibroblasts, and MSCs died off in PEGDA hydrogels over two weeks. PEGNB hydrogels, however, can better support long-term cell survival. Fibroblasts, in particular, showed a significant preference to PEGNB hydrogels following either bulk or microfluidic encapsulation. β -cells failed to display long-term survival under all tested conditions (Fig. 7).

To understand variable cell tolerance to the encapsulants employed, viability results from PEGNB were taken as standards, and cell viability in PEGDA hydrogels on day 1 and day 14 were normalized to that of corresponding PEGNB hydrogels. It was observed that even though slightly decreased, A549s, fibroblasts, and MSCs in PEGDA bulk or micro gels can maintain a high initial viability, similar to the results from PEGNB. However, after two weeks in culture, cell viability decreased tremendously for all the cells encapsulated in PEGDA bulk hydrogels, and even more severely in PEGDA microgels, indicating that given currently available droplet microencapsulation strategies, PEGNB is universally preferred. Even though β -cells had a high viability in PEGDA bulk hydrogels initially, this transient result did not persist (Fig. 8).

Comparing results between two encapsulation schemes by taking bulk encapsulation as a standard, we found that there was no obvious viability change in A549s and MSCs on day 1 in either PEGDA or PEGNB hydrogels (Fig. 9(a)). Fibroblasts, however, displayed decreased viability on day 1, but this reduction was not further exaggerated over two weeks in culture (Fig. 9(b)). When comparing the tremendous difference observed for β -cells following their bulk and micro encapsulation in PEGDA, and considering that encapsulation strategy is the only differentiating factor, it is clear that hydrogel miniaturization is not as straightforward as shrinking the physical dimensions of hydrogels. Many factors must be considered to optimize the cytocompatibility of microencapsulation platforms. For example, while microfluidic droplet encapsulation in photopolymerized PEGNB has been verified as cell-friendly[88], exaggerated levels of cumulative ROS within PEGDA microgels following droplet photopolymerization in oxygen-abundant fluorocarbon oils clearly play a crucial role in reducing cell viability. Our nitrogen purging device prevents nascent oxygen from

diffusing to the emulsion through the PDMS but cannot eliminate extant oxygen in the droplet or oil phases. More effective transient oxygen removal approaches should be further investigated to improve the cytocompatibility of cell microencapsulation platform when using PEGDA.

In general, constrained by current understanding of cell-material interactions and available miniaturization techniques, bulk encapsulation provides higher overall cell viability than microencapsulation. PEGNB, however, with its still-unresolved materials chemistry, has the potential to completely negate the negative cellular response to high-throughput droplet-based microgel encapsulation.

Conclusions

In this study, we have demonstrated the versatility and cytocompatibility of a previously introduced microfluidic-based cell microencapsulation platform employing PEGNB as a cell encapsulant. Experimental parameters including UV irradiation intensity, time, photoinitiator concentration, and hydrogel surface area have been investigated and optimized for cytocompatibility. We observed that cells encapsulated into PEGNB hydrogels can tolerate the UV intensity and exposure time used in these experiments. Although no harmful effects have been observed at higher UV intensities, longer exposure time for PEGDA hydrogels can cause dramatic damage in cells. Increased level of photoinitiator can post deleterious effects on cells in a concentration dependent manner for both materials. The deleterious effects of increased time and initiator concentration are consistent with the hypothesis linking cell death with elevated ROS accumulation during photopolymerization. The increase in hydrogel surface area is proportional to the decrease in cell viability in PEGDA, but can be mitigated in PEGNB, using equivalent photoencapsulation parameters. Encapsulating four mammalian cell candidates into bulk or microgel PEGNB and PEGDA with varying macromer concentrations has revealed variable cellular response to encapsulation. PEGNB material chemistry, coupling with superior polymerization kinetics, has contributed to an enhanced and sustainable cell survival after encapsulation for most of the cell types. Increasing macromer concentration would compromise long-term cell survival, presumably because the increase in hydrogel stiffness and decrease in mesh size does not permit sufficient solute transport to and from cells. In conclusion, we demonstrate our microfluidic-based PEGNB microgel fabrication platform is capable of producing highly monodisperse cell-laden microgels in a high throughput manner, with excellent cytocompatibility and versatility. The feasibility of cell encapsulation on the microscale, combined with the potential for hydrogel modification with bioligands, can support this cell microencapsulation platform to be broadly used for bottom-up tissue engineering and therapeutic applications.

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References

- [1]. Yang SF, Leong KF, Du ZH and Chua CK 2001 The design of scaffolds for use in tissue engineering. Part 1. Traditional factors Tissue Engineering 7 679–89 [PubMed: 11749726]
- [2]. Hollister SJ 2005 Porous scaffold design for tissue engineering Nature Materials 4 518–24 [PubMed: 16003400]
- [3]. Caplan AI 2007 Adult mesenchymal stem cells for tissue engineering versus regenerative medicine J. Cell. Physiol 213 341–7 [PubMed: 17620285]
- [4]. Wong T, McGrath JA and Navsaria H 2007 The role of fibroblasts in tissue engineering and regeneration Br J Dermatol 156 1149–55 [PubMed: 17535219]
- [5]. Singh SP, Schwartz MP, Tokuda EY, Luo Y, Rogers RE, Fujita M, Ahn NG and Anseth KS 2015 A synthetic modular approach for modeling the role of the 3D microenvironment in tumor progression Sci. Rep 1–9
- [6]. Asghar W, Assal El R, Shafiee H, Pitteri S, Paulmurugan R and Demirci U 2015 Engineering cancer microenvironments for in vitro 3-D tumor models Biochemical Pharmacology 18 539–53
- [7]. Drury JL and Mooney DJ 2003 Hydrogels for tissue engineering: scaffold design variables and applications Biomaterials 24 4337–51 [PubMed: 12922147]
- [8]. Griffith LG and Naughton G 2002 Tissue engineering - Current challenges and expanding opportunities Science 295 1009–1014 [PubMed: 11834815]
- [9]. Langer R 2009 Perspectives and Challenges in Tissue Engineering and Regenerative Medicine Adv. Mater 21 3235–6 [PubMed: 20882493]
- [10]. Parratt K, Smerchansky M, Stiggers Q and Roy K 2017 Effect of hydrogel material composition on hBMSC differentiation into zone-specific neo-cartilage: engineering human articular cartilage-like tissue with spatially varying properties Journal of Materials Chemistry B: Materials for biology and medicine 1–12
- [11]. Orive G, Maria Hernandez R, Gascon AR, Calafiore R, Chang TMS, De Vos P, Hortelano G, Hunkeler D, Lacik I, Shapiro AMJ and Luis Pedraz J 2003 Cell encapsulation: Promise and progress Nat Med 9 104–7 [PubMed: 12514721]
- [12]. Wang Q, Mynar JL, Yoshida M, Lee E, Lee M, Okuro K, Kinbara K and Aida T 2010 High-water-content mouldable hydrogels by mixing clay and a dendritic molecular binder Nature 463 339–43 [PubMed: 20090750]
- [13]. Amsden B 1998 Solute Diffusion within Hydrogels. Mechanisms and Models Macromolecules 31 8382–95
- [14]. Lacy PE, Hegre OD, Gerasimidi-Vazeou A, Gentile FT and Dionne KE 1991 Maintenance of normoglycemia in diabetic mice by subcutaneous xenografts of encapsulated islets Science 254 1782–4 [PubMed: 1763328]
- [15]. Desai TA, Chu WH, Rasi G, Sinibaldi-Vallebona P, Guarino E and Ferrari M 1999 Microfabricated Biocapsules Provide Short-Term Immunoisolation of Insulinoma Xenografts Biomed Microdevices 1 131–8 [PubMed: 16281113]
- [16]. Carlo DD, Wu LY and Lee LP 2006 Dynamic single cell culture array Lab Chip 6 1445–5 [PubMed: 17066168]
- [17]. Vickerman V, Blundo J, Chung S and Kamm R 2008 Design, fabrication and implementation of a novel multi-parameter control microfluidic platform for three-dimensional cell culture and real-time imaging Lab Chip 8 1468–10 [PubMed: 18818801]
- [18]. Yang F, Williams CG, Wang D-A, Lee H, Manson PN and Elisseeff J 2005 The effect of incorporating RGD adhesive peptide in polyethylene glycol diacrylate hydrogel on osteogenesis of bone marrow stromal cells Biomaterials 26 5991–8 [PubMed: 15878198]
- [19]. Desimone MF, Hélaré C, Mosser G, Giraud-Guille M-M, Livage J and Coradin T 2010 Fibroblast encapsulation in hybrid silica-collagen hydrogels J. Mater. Chem 20 666–8
- [20]. Weber LM, Hayda KN, Haskins K and Anseth KS 2007 The effects of cell-matrix interactions on encapsulated β -cell function within hydrogels functionalized with matrix-derived adhesive peptides Biomaterials 28 3004–11 [PubMed: 17391752]

- [21]. Lin C-C and Anseth KS 2009 Glucagon-Like Peptide-1 Functionalized PEG Hydrogels Promote Survival and Function of Encapsulated Pancreatic β -Cells Biomacromolecules 10 2460–7 [PubMed: 19586041]
- [22]. Hwang NS, Varghese S, Theprungsirikul P, Canver A and Elisseeff J 2006 Enhanced chondrogenic differentiation of murine embryonic stem cells in hydrogels with glucosamine Biomaterials 27 6015–23 [PubMed: 16872674]
- [23]. DeLong SA, Moon JJ and West JL 2005 Covalently immobilized gradients of bFGF on hydrogel scaffolds for directed cell migration Biomaterials 26 3227–34 [PubMed: 15603817]
- [24]. Nikravesh N, Cox SC, Ellis MJ and Grover LM 2017 Encapsulation and Fluidization Maintains the Viability and Glucose Sensitivity of Beta-Cells ACS Biomater. Sci. Eng 3 1750–7
- [25]. Selmi T, Verdonk P and Chambat P 2008 Autologous chondrocyte implantation in a novel alginate-agarose hydrogel Journal of Bone & Joint Surgery
- [26]. Lim F and Sun AM 1980 Microencapsulated Islets as Bioartificial Endocrine Pancreas Science 210 908–10 [PubMed: 6776628]
- [27]. Nuttelman CR, Benoit DSW, Tripodi MC and Anseth KS 2006 The effect of ethylene glycol methacrylate phosphate in PEG hydrogels on mineralization and viability of encapsulated hMSCs Biomaterials 27 1377–86 [PubMed: 16139351]
- [28]. Yang J-A, Yeom J, Hwang BW, Hoffman AS and Hahn SK 2014 In situ-forming injectable hydrogels for regenerative medicine Progress in Polymer Science 39 1973–86
- [29]. Tan H, Chu CR, Payne KA and Marra KG 2009 Injectable in situ forming biodegradable chitosan-hyaluronic acid based hydrogels for cartilage tissue engineering Biomaterials 30 2499–506 [PubMed: 19167750]
- [30]. Uludag H, De Vos P and Tresco PA 2000 Technology of mammalian cell encapsulation Advanced Drug Delivery Reviews 42 29–64 [PubMed: 10942814]
- [31]. Wang J-Z, Ding Z-Q, Zhang F and Ye W-B 2017 Recent development in cell encapsulations and their therapeutic applications Materials Science & Engineering C 1–14
- [32]. Wang L, Qiu M, Yang Q, Li Y, Huang G, Lin M, Lu TJ and Xu F 2015 Fabrication of Microscale Hydrogels with Tailored Microstructures based on Liquid Bridge Phenomenon ACS Appl. Mater. Interfaces 7 11134–40 [PubMed: 25726861]
- [33]. Hwang DK, Oakey J, Toner M, Arthur JA, Anseth KS, Lee S, Zeiger A, Van Vliet KJ and Doyle PS 2009 Stop-Flow Lithography for the Production of Shape-Evolving Degradable Microgel Particles J. Am. Chem. Soc 131 4499–504 [PubMed: 19215127]
- [34]. Ma Y, Ji Y, Huang G, Ling K, Zhang X and Xu F 2015 Bioprinting 3D cell-laden hydrogel microarray for screening human periodontal ligament stem cell response to extracellular matrix Biofabrication 7 1–9
- [35]. Su Y-H, Chiang P-C, Cheng L-J, Lee C-H, Swami NS and Chou C-F 2015 High aspect ratio nanoimprinted grooves of poly(lactic-co-glycolic acid) control the length and direction of retraction fibers during fibroblast cell division Biointerphases 10 041008–9 [PubMed: 26652706]
- [36]. Griffin DR and Kasko AM 2012 Photodegradable Macromers and Hydrogels for Live Cell Encapsulation and Release J. Am. Chem. Soc 134 13103–7 [PubMed: 22765384]
- [37]. Maltzahn von G, Min DH, Zhang Y, Park JH, Harris TJ, Sailor M and Bhatia SN 2007 Nanoparticle Self-Assembly Directed by Antagonistic Kinase and Phosphatase Activities Adv. Mater 19 3579–83
- [38]. Choi C-H, Wang H, Lee H, Kim JH, Zhang L, Mao A, Mooney DJ and Weitz DA 2016 One-step generation of cell-laden microgels using double emulsion drops with a sacrificial ultra-thin oil shell Lab Chip 16 1549–55 [PubMed: 27070224]
- [39]. Grigore A, Sarker B, Fabry B, Boccaccini AR and Detsch R 2014 Behavior of Encapsulated MG-63 Cells in RGD and Gelatine-Modified Alginate Hydrogels Tissue Engineering Part A 20 2140–50 [PubMed: 24813329]
- [40]. Kesselman LRB, Shinwary S, Selvaganapathy PR and Hoare T 2012 Synthesis of Monodisperse, Covalently Cross-Linked, Degradable “Smart” Microgels Using Microfluidics Small 8 1092–8 [PubMed: 22354786]

- [41]. Tan Y-C, Hettiarachchi K, Siu M, Pan Y-R and Lee AP 2006 Controlled Microfluidic Encapsulation of Cells, Proteins, and Microbeads in Lipid Vesicles *J. Am. Chem. Soc.* 128 5656–8 [PubMed: 16637631]
- [42]. Griffin DR, Weaver WM, Scumpia PO, Di Carlo D and Segura T 2015 Accelerated wound healing by injectable microporous gel scaffolds assembled from annealed building blocks *Nature Materials* 14 737–44 [PubMed: 26030305]
- [43]. Deveza L, Ashoken J, Castaneda G, Tong X, Keeney M, Han L-H and Yang F 2015 Microfluidic Synthesis of Biodegradable Polyethylene-Glycol Microspheres for Controlled Delivery of Proteins and DNA Nanoparticles *ACS Biomater. Sci. Eng.* 1 157–65
- [44]. Kumachev A, Greener J, Tumarkin E, Eiser E, Zandstra PW and Kumacheva E 2011 High-throughput generation of hydrogel microbeads with varying elasticity for cell encapsulation *Biomaterials* 32 1477–83 [PubMed: 21095000]
- [45]. Lu Y-C, Song W, An D, Kim BJ, Schwartz R, Wu M and Ma M 2014 Designing compartmentalized hydrogel microparticles for cell encapsulation and scalable 3D cell culture *Journal of Materials Chemistry B: Materials for biology and medicine* 3 353–60
- [46]. Reece AE and Oakey J 2016 Long-range forces affecting equilibrium inertial focusing behavior in straight high aspect ratio microfluidic channels *Phys. Fluids* 28 043303–11
- [47]. Oakey J, Applegate RW, Jr., Arellano E, Carlo DD, Graves SW and Toner M 2010 Particle Focusing in Staged Inertial Microfluidic Devices for Flow Cytometry *Anal. Chem.* 82 3862–7 [PubMed: 20373755]
- [48]. Edd JF, Di Carlo D, Humphry KJ, Köster S, Irimia D, Weitz DA and Toner M 2008 Controlled encapsulation of single-cells into monodisperse picolitre drops *Lab Chip* 8 1262–3 [PubMed: 18651066]
- [49]. Reece A, Xia B, Jiang Z, Noren B, McBride R and Oakey J 2016 ScienceDirect Microfluidic techniques for high throughput single cell analysis *Current Opinion in Biotechnology* 40 90–6 [PubMed: 27032065]
- [50]. Clausell-Tormos J, Lieber D, Baret J-C, El-Harrak A, Miller OJ, Frenz L, Blouwolff J, Humphry KJ, Köster S, Duan H, Holtze C, Weitz DA, Griffiths AD and Merten CA 2008 Droplet-Based Microfluidic Platforms for the Encapsulation and Screening of Mammalian Cells and Multicellular Organisms *Chemistry & Biology* 15 427–37 [PubMed: 18482695]
- [51]. Brouzes E, Medkova M, Savenelli N, Marran D, Twardowski M, Hutchison JB, Rothberg JM, Link DR, Perrimon N and Samuels ML 2009 Droplet microfluidic technology for single-cell high-throughput screening *Proc Natl Acad Sci USA* 106 14195–200 [PubMed: 19617544]
- [52]. Klein AM, Mazutis L, Akartuna I, Tallapragada N, Veres A, Li V, Peshkin L, Weitz DA and Kirschner MW 2015 Droplet Barcoding for Single-Cell Transcriptomics Applied to Embryonic Stem Cells *Cell* 161 1187–201 [PubMed: 26000487]
- [53]. Macosko EZ, Basu A, Satija R, Nemesh J, Shekhar K, Goldman M, Tirosh I, Bialas AR, Kamitaki N, Martersteck EM, Trombetta JJ, Weitz DA, Sanes JR, Shalek AK, Regev A and McCarroll SA 2015 Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets *Cell* 161 1202–14 [PubMed: 26000488]
- [54]. Velasco D, Tumarkin E and Kumacheva E 2012 Microfluidic Encapsulation of Cells in Polymer Microgels *Small* 8 1633–42 [PubMed: 22467645]
- [55]. Xia B, Krutkramelis K and Oakey J 2016 Oxygen-Purged Microfluidic Device to Enhance Cell Viability in Photopolymerized PEG Hydrogel Microparticles *Biomacromolecules* 17 2459–65 [PubMed: 27285343]
- [56]. Tan WH and Takeuchi S 2007 Monodisperse Alginate Hydrogel Microbeads for Cell Encapsulation *Adv. Mater.* 19 2696–701
- [57]. Tumarkin E and Kumacheva E 2009 Microfluidic generation of microgels from synthetic and natural polymers *Chem. Soc. Rev.* 38 2161–9 [PubMed: 19623340]
- [58]. Jiang W, Li M, Chen Z and Leong KW 2016 Cell-laden microfluidic microgels for tissue regeneration *Lab Chip* 16 4482–506 [PubMed: 27797383]
- [59]. Nichol JW, Koshy ST, Bae H, Hwang CM, Yamanlar S and Khademhosseini A 2010 Cell-laden microengineered gelatin methacrylate hydrogels *Biomaterials* 31 5536–44 [PubMed: 20417964]

- [60]. Langer R and Tirrell DA 2004 Designing materials for biology and medicine *Nature* 428 487–92 [PubMed: 15057821]
- [61]. Allazetta S, Hausherr TC and Lutolf MP 2013 Microfluidic Synthesis of Cell-Type-Specific Artificial Extracellular Matrix Hydrogels *Biomacromolecules* 14 1122–31 [PubMed: 23439131]
- [62]. Rane AA, Chuang JS, Shah A, Hu DP, Dalton ND, Gu Y, Peterson KL, Omens JH and Christman KL 2011 Increased Infarct Wall Thickness by a Bio-Inert Material Is Insufficient to Prevent Negative Left Ventricular Remodeling after Myocardial Infarction *ed W-C Chin PLoS ONE* 6 e21571–8 [PubMed: 21731777]
- [63]. Weber LM, Lopez CG and Anseth KS 2009 Effects of PEG hydrogel crosslinking density on protein diffusion and encapsulated islet survival and function *J. Biomed. Mater. Res* 90A 720–9
- [64]. Kloxin AM, Kasko AM, Salinas CN and Anseth KS 2009 Photodegradable Hydrogels for Dynamic Tuning of Physical and Chemical Properties *Science* 324 59–63 [PubMed: 19342581]
- [65]. Schmidt JJ, Rowley J and Kong HJ 2008 Hydrogels used for cell-based drug delivery *J. Biomed. Mater. Res* 87A 1113–22
- [66]. Lutolf MP and Hubbell JA 2005 Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering *Nat Biotechnol* 23 47–55 [PubMed: 15637621]
- [67]. Headen DM, Aubry G, Lu H and García AJ 2014 Microfluidic-Based Generation of Size-Controlled, Biofunctionalized Synthetic Polymer Microgels for Cell Encapsulation *Adv. Mater* 26 3003–8 [PubMed: 24615922]
- [68]. Nicodemus GD and Bryant SJ 2008 Cell Encapsulation in Biodegradable Hydrogels for Tissue Engineering Applications *Tissue Engineering Part B: Reviews* 14 149–65 [PubMed: 18498217]
- [69]. Tibbitt MW, Kloxin AM, Sawicki LA and Anseth KS 2013 Mechanical Properties and Degradation of Chain and Step-Polymerized Photodegradable Hydrogels *Macromolecules* 46 2785–92
- [70]. Kloxin AM, Kloxin CJ, Bowman CN and Anseth KS 2009 Mechanical Properties of Cellularly Responsive Hydrogels and Their Experimental Determination *Adv. Mater* 22 3484–94
- [71]. Tong X, Jiang J, Zhu D and Yang F 2016 Hydrogels with Dual Gradients of Mechanical and Biochemical Cues for Deciphering Cell-Niche Interactions *ACS Biomater. Sci. Eng* 2 845–52
- [72]. Yang C, DelRio FW, Ma H, Killaars AR, Basta LP, Kyburz KA and Anseth KS 2016 Spatially patterned matrix elasticity directs stem cell fate *Proc Natl Acad Sci USA* 113 E4439–45 [PubMed: 27436901]
- [73]. Van Tomme SR, Storm G and Hennink WE 2008 In situ gelling hydrogels for pharmaceutical and biomedical applications *International Journal of Pharmaceutics* 355 1–18 [PubMed: 18343058]
- [74]. Roberts JJ and Bryant SJ 2013 Comparison of photopolymerizable thiol-ene PEG and acrylate-based PEG hydrogels for cartilage development *Biomaterials* 34 9969–79 [PubMed: 24060418]
- [75]. Burdick JA and Anseth KS 2002 Photoencapsulation of osteoblasts in injectable RGD-modified PEG hydrogels for bone tissue engineering *Biomaterials* 23 4315–23 [PubMed: 12219821]
- [76]. Chan V, Zorlutuna P, Jeong JH, Kong H and Bashir R 2010 Three-dimensional photopatterning of hydrogels using stereolithography for long-term cell encapsulation *Lab Chip* 10 2062–10 [PubMed: 20603661]
- [77]. Siltanen C, Yaghoobi M, Haque A, You J, Lowen J, Soleimani M and Revzin A 2016 Microfluidic fabrication of bioactive microgels for rapid formation and enhanced differentiation of stem cell spheroids *ACTA BIOMATERIALIA* 1–8
- [78]. Lin C-C, Metters AT and Anseth KS 2009 Functional PEG-peptide hydrogels to modulate local inflammation induced by the pro-inflammatory cytokine TNF α ; *Biomaterials* 30 4907–14 [PubMed: 19560813]
- [79]. Shenoy R and Bowman CN 2010 Mechanism and Implementation of Oxygen Inhibition Suppression in Photopolymerizations by Competitive Photoactivation of a Singlet Oxygen Sensitizer *Macromolecules* 43 7964–70
- [80]. Lin C-C, Raza A and Shih H 2011 PEG hydrogels formed by thiol-ene photo-click chemistry and their effect on the formation and recovery of insulin-secreting cell spheroids *Biomaterials* 32 9685–95 [PubMed: 21924490]

- [81]. Desai ES, Tang MY, Ross AE and Gemeinhart RA 2012 Critical factors affecting cell encapsulation in superporous hydrogels *Biomed. Mater* 7
- [82]. Ke C, Sun L, Qiao D, Wang Di and Zeng X 2011 Antioxidant activity of low molecular weight hyaluronic acid *Food and Chemical Toxicology* 49 2670–5 [PubMed: 21787831]
- [83]. Sato H, Takahashi T, Ide H, Fukushima T, Tabata M, Sekine F, Kobayashi K, Negishi M and Niwa Y 1988 Antioxidant activity of synovial fluid, hyaluronic acid, and two subcomponents of hyaluronic acid. synovial fluid scavenging effect is enhanced in rheumatoid arthritis patients *Arthritis & Rheumatism* 31 63–71 [PubMed: 3345232]
- [84]. Gramlich WM, Kim IL and Burdick JA 2013 Synthesis and orthogonal photopatterning of hyaluronic acid hydrogels with thiol-norbornene chemistry *Biomaterials* 34 9803–11 [PubMed: 24060422]
- [85]. Fairbanks BD, Schwartz MP, Halevi AE, Nuttelman CR, Bowman CN and Anseth KS 2009 A Versatile Synthetic Extracellular Matrix Mimic via Thiol- Norbornene Photopolymerization *Adv. Mater* 21 5005–10 [PubMed: 25377720]
- [86]. Tokuda EY, Leight JL and Anseth KS 2014 Modulation of matrix elasticity with PEG hydrogels to study melanoma drug responsiveness *Biomaterials* 35 4310–8 [PubMed: 24565518]
- [87]. O'Brien AK, Cramer NB and Bowman CN 2006 Oxygen inhibition in thiol-acrylate photopolymerizations *J. Polym. Sci. A Polym. Chem* 44 2007–14
- [88]. Jiang Z, Xia B, McBride R and Oakey J 2017 A microfluidic-based cell encapsulation platform to achieve high long-term cell viability in photopolymerized PEGNB hydrogel microspheres *Journal of Materials Chemistry B: Materials for biology and medicine* 5 173–80 [PubMed: 28066550]
- [89]. Rossow T, Heyman JA, Ehrlicher AJ, Langhoff A, Weitz DA, Haag R and Seiffert S 2012 Controlled Synthesis of Cell-Laden Microgels by Radical-Free Gelation in Droplet Microfluidics *J. Am. Chem. Soc* 134 4983–9 [PubMed: 22356466]
- [90]. Williams CG, Malik AN, Kim TK, Manson PN and Elisseeff JH 2005 Variable cytocompatibility of six cell lines with photoinitiators used for polymerizing hydrogels and cell encapsulation *Biomaterials* 26 1211–8 [PubMed: 15475050]
- [91]. Li CY, Wood DK, Huang JH and Bhatia SN 2013 Flow-based pipeline for systematic modulation and analysis of 3D tumor microenvironments *Lab Chip* 13 1969–12 [PubMed: 23563587]
- [92]. Krutkramelis K, Xia B and Oakey J 2016 Monodisperse polyethylene glycol diacrylate hydrogel microsphere formation by oxygen-controlled photopolymerization in a microfluidic device *Lab Chip* 1–9
- [93]. Lin C-C, Ki CS and Shih H 2014 Thiol-norbornene photoclick hydrogels for tissue engineering applications *J. Appl. Polym. Sci* 132 (8):41563
- [94]. Fairbanks BD, Schwartz MP, Bowman CN and Anseth KS 2009 Photoinitiated polymerization of PEG-diacrylate with lithium phenyl-2,4,6-trimethylbenzoylphosphinate: polymerization rate and cytocompatibility *Biomaterials* 30 6702–7 [PubMed: 19783300]
- [95]. Anderson SB, Lin C-C, Kuntzler DV and Anseth KS 2011 The performance of human mesenchymal stem cells encapsulated in cell-degradable polymer-peptide hydrogels *Biomaterials* 32 3564–74 [PubMed: 21334063]
- [96]. Shih H and Lin C-C 2012 Cross-Linking and Degradation of Step-Growth Hydrogels Formed by Thiol-Ene Photoclick Chemistry *Biomacromolecules* 13 2003–12 [PubMed: 22708824]
- [97]. Majima T, Schnabel W and Weber W 1991 Phenyl-2,4,6- trimethylbenzoylphosphinates as water-soluble photoinitiators. Generation and reactivity of O=P(C6H5)(O-) radical anions *Die Makromolekulare Chemie* 192 2307–15
- [98]. Xia Y and Whitesides GM 1998 Soft lithography *Annual review of materials science* 28 153–84
- [99]. Jain E, Hill L, Canning E, Sell SA and Zustiak SP 2017 Control of gelation, degradation and physical properties of polyethylene glycol hydrogels through the chemical and physical identity of the crosslinker *Journal of Materials Chemistry B: Materials for biology and medicine* 5 2679–91
- [100]. Chatterjee K, Lin-Gibson S, Wallace WE, Parekh SH, Lee YJ, Cicerone MT, Young MF and Simon CG, Jr 2010 The effect of 3D hydrogel scaffold modulus on osteoblast differentiation and

mineralization revealed by combinatorial screening *Biomaterials* 31 5051–62 [PubMed: 20378163]

- [101]. Ryu S, Kim HH, Park YH, Lin CC, Um IC and Ki CS 2016 Dual mode gelation behavior of silk fibroin microgel embedded poly(ethylene glycol) hydrogels *Journal of Materials Chemistry B: Materials for biology and medicine* 4 4574–84
- [102]. Xia B, Jiang Z, Debroy D, Li D and Oakey J 2017 Cytocompatible cell encapsulation via hydrogel photopolymerization in microfluidic emulsion droplets *Biomicrofluidics* 11 044102–11 [PubMed: 28794813]
- [103]. Dendukuri D, Panda P, Haghighooie R, Kim JM, Hatton TA and Doyle PS 2008 Modeling of Oxygen-Inhibited Free Radical Photopolymerization in a PDMS Microfluidic Device *Macromolecules* 41 8547–56
- [104]. Andrzejewska E 2001 Photopolymerization kinetics of multifunctional monomers *Progress in Polymer Science* 26 605–65
- [105]. Wang T, Lai JH, Han L-H, Tong X and Yang F 2016 Modulating stem cell-chondrocyte interactions for cartilage repair using combinatorial extracellular matrix-containing hydrogels *Journal of Materials Chemistry B: Materials for biology and medicine* 4 7641–7650
- [106]. Shih H, Liu H-Y and Lin C-C 2017 Improving gelation efficiency and cytocompatibility of visible light polymerized thiol-norbornene hydrogels via addition of soluble tyrosine *Biomaterials Science* 5 589–599 [PubMed: 28174779]
- [107]. McCall JD and Anseth KS 2012 Thiol-Ene Photopolymerizations Provide a Facile Method To Encapsulate Proteins and Maintain Their Bioactivity *Biomacromolecules* 13 2410–7 [PubMed: 22741550]
- [108]. Mi Y, Chan Y, Trau D, Huang P and Chen E 2006 Micromolding of PDMS scaffolds and microwells for tissue culture and cell patterning: A new method of microfabrication by the self-assembled micropatterns of diblock copolymer micelles *Polymer* 47 5124–30
- [109]. Cuchiara MP, Allen ACB, Chen TM, Miller JS and West JL 2010 Multilayer microfluidic PEGDA hydrogels *Biomaterials* 31 5491–7 [PubMed: 20447685]
- [110]. Stowers RS, Allen SC and Suggs LJ 2015 Dynamic phototuning of 3D hydrogel stiffness *Proc Natl Acad Sci USA* 112 1953–8 [PubMed: 25646417]
- [111]. Wen JH, Vincent LG, Fuhrmann A, Choi YS, Hribar KC, Taylor-Weiner H, Chen S and Engler AJ 2014 Interplay of matrix stiffness and protein tethering in stem cell differentiation *Nature Materials* 13 979–87 [PubMed: 25108614]
- [112]. Caliri SR, Perepelyuk M, Cosgrove BD, Tsai SJ, Lee GY, Mauck RL, Wells RG and Burdick JA 2016 Stiffening hydrogels for investigating the dynamics of hepatic stellate cell mechanotransduction during myofibroblast activation *Sci. Rep* 1–10 [PubMed: 28442746]
- [113]. Liao H, Munoz-Pinto D, Qu X, Hou Y, Grunlan MA and Hahn MS 2008 Influence of hydrogel mechanical properties and mesh size on vocal fold fibroblast extracellular matrix production and phenotype *ACTA BIOMATERIALIA* 4 1161–71 [PubMed: 18515199]
- [114]. Lin CC and Anseth KS 2011 Cell-cell communication mimicry with poly (ethylene glycol) hydrogels for enhancing β -cell function *Pro Natl Acad Sci USA* 108 6380–6385
- [115]. Weber LM, He J, Bradley B, Haskins K and Anseth KS 2006 PEG-based hydrogels as an in vitro encapsulation platform for testing controlled β -cell microenvironments *ACTA BIOMATERIALIA* 2 1–8 [PubMed: 16701853]
- [116]. Anseth KS, Metters AT, Bryant SJ, Martens PJ, Elisseeff JH and Bowman CN 2002 In situ forming degradable networks and their application in tissue engineering and drug delivery *Journal of Controlled Release* 78 199–209 [PubMed: 11772461]
- [117]. McKinnon DD, Kloxin AM and Anseth KS 2013 Synthetic hydrogel platform for three-dimensional culture of embryonic stem cell-derived motor neurons *Biomaterials Science* 1 460–11

Fig 1. Schematic of cell encapsulation within PEGNB or PEGDA hydrogel forming solutions via either flow-focusing (a) or cross-flow microfluidic channels (b). Once formed, PEGNB droplets are collected for off-chip batch photopolymerization (a) or polymerized on-chip by UV exposure while flowing through a double layer nitrogen jacketed channel device (b).

Fig 2.

(a) Fluorescence images, and (b) size distribution of PEGNB/PEGDA microgels produced by photopolymerizing microfluidically generated droplets over a range of relative aqueous:oil flow rate ratios. Cell-laden PEGNB microgels (c) are fluorescently labeled with Rhodamine B. Scale bar, 200 μm.

Fig 3.

Cell tolerance to variable photopolymerization parameters under bulk conditions. (a) A schematic of bulk photopolymerization in which cells are encapsulated within macroscale PEG hydrogels. (b) Cell viability in PEGNB or PEGDA hydrogels following photopolymerization under varying UV intensity while holding exposure time constant at 20 seconds. (c) Cell viability in PEGNB or PEGDA hydrogels with varying UV exposure times while holding intensity constant at 100 mW/cm^2 . (d) Cell viability in PEGNB or PEGDA hydrogels formed under UV exposure at 100 mW/cm^2 for 20 seconds with varying photoinitiator concentrations.

Fig 4. Cell response to changes in hydrogel surface area. (a) Fluorescent images of A549s encapsulated within 10 wt% PEGDA or PEGNB hydrogel discs or hydrogel thin films. Scale bar, 100 μ m (b) Quantified initial cell viability of different polymerization schemes.

Fig 5. Cell-dependent tolerance to encapsulation into PEGNB or PEGDA bulk hydrogel discs with varying macromer concentrations. (a) A549, (b) 3T3 fibroblast, (c) MSC, (d) β -cell.

Fig 6. Cell-dependent tolerance to microfluidic encapsulation into PEGNB or PEGDA microgels with varying macromer concentrations. (a) A549, (b) 3T3 fibroblast, (c) MSC, (d) β -cell.

Fig 7. Response of 4 cell types to encapsulation into 10 wt % PEGDA/PEGNB bulk or microgels over 2-week period. Given cell viability is 0% in PEGDA microgels on day 14, results are normalized to a 100% decrease from day 1.

Fig 8.

Relative performance of 4 cell types to encapsulation into 10 wt % PEGDA bulk or microgels on day 1 and day 14, normalized to corresponding PEGNB gels. On day 14, cell viability in PEGDA microgels, which is 0% for all the cases, is considered as 100% decrease compared to that in PEGNB.

Fig 9.

Relative response of 4 cell types to micro encapsulation into 10 wt % PEGDA or PEGNB microgels on day 1 and day 14, which is normalized to corresponding bulk gels. Cell viability in PEGDA microgels on day 14 is considered as 100% decrease from that in bulk gel.