Viral infection of human progenitor and liver-derived cells encapsulated in three-dimensional PEG-based hydrogel

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Supporting Information Content

Optimization of cell encapsulation in PEG8k hydrogels

Swelling measurements of different molecular weights of poly(ethylene glycol) diacrylate hydrogel with and without encapsulating Huh-7.5 cells at 2.5x10\textsuperscript{6} cells/ml

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Comparison between tensile stress and tensile strain tension for PEG-DA hydrogels of different molecular weights
**Optimization of cell encapsulation in PEG8k hydrogels:**

Chemically cross-linked hydrogel networks are driven by chemicals that produce free radicals when exposed to specific wavelengths of light. Free radicals can damage cell membranes, nucleic acids, and proteins and may lead to cell death. Moreover, oxidative damage may vary depending on cell type and proliferation rate. Several groups have made these systems cytocompatible and useful for in vitro and in vivo tissue engineering applications. However, it is necessary to optimize the cytocompatibility conditions depending on cell types of interest. In order to determine optimal conditions for encapsulating Huh7.5 cells in the hydrogel, we first investigated cell viability as a function of a variety of parameters including UV intensity and exposure time, photoinitiator, and different molecular weight-based PEGs. Optimal viability was obtained with PEG 8k diacrylate hydrogels, and we used this PEG for our studies.

We chose the photoinitiator (P.I.), Irgacure 2959 (I-2959, Ciba), to cross link the network and determined the cytocompatible condition for the photoinitiator to achieve Huh7.5 cell encapsulation. We found that the final concentration of the photoinitiator throughout the experiment should be 0.05% (w/v), as shown in Figure S1-1. It is worth noting that a higher photoinitiator (~ 1%) significantly decreased cell viability.

In order to determine the toxicity of UV exposure time and intensity, we altered the intensity of the UV by changing distance and time. As shown in Figure S1-2, the cell viability remained greater than 90% for 10 mW/cm² up to one min. However, exposure for 10 min decreased cell viability by 50%. These features are more dramatic as the UV intensity is increased. Throughout subsequent experiments, we chose a period of one minute for UV exposure with an intensity of 10 mW/cm². We next combined the effects of P.I. and UV, as shown in Figure S1-3. The combination of P.I. dissolved in water (~0.05%) and a UV intensity of 10 mW/cm² showed good cell viability following UV exposure of up to two minutes. Because good polymerization was observed at one minute, we continued the Huh7.5 cell encapsulating investigation with different molecular weights of the PEG-DA using this optimized condition (P.I. ~ 0.05%, UV ~ one min, 10 mW/cm²). As we anticipated (Figure S1-4), PEG-DAs of larger molecular weights (3.4k, 8k) resulted in good cell viability for up to 14 days. However, PEG-DA 700 demonstrated poor cell viability.
Figure S1-1
Huh7.5 cell viability following exposure to different photoinitiator concentrations.
Figure S1-2

Huh7.5 cell viability following exposure to different UV intensity and exposure time.
Figure S1-3
Huh7.5 cell viability when exposed to combined UV and photoinitiator (P.I.). Cells were maintained in cell culture medium without P.I. (○), 0.05%(w/v) P.I. that was dissolved in water (●) and 0.05%(w/v) P.I. that was dissolved in methanol (■) UV intensity was 10 mW/cm² for all conditions.
Figure S1-4
Cell viability of encapsulated Huh7.5 cells for different PEG-DA molecular weights.
Swelling measurements of different molecular weights of poly(ethylene glycol) diacrylate hydrogel with and without encapsulating Huh-7.5 cells at 2.5x10^6 cells/ml

The swollen-weight-to-dry-weight ratio of water content with and without encapsulating cells did not exhibit any significant difference. The 20 wt% 700 PEG-DA showed a water content between 65 - 70%. The 10 wt% to 50 wt% of 8k PEG-DA exhibited water content near ~ 90%.

Figure S2

The water content of different molecular weights of PEG-DA hydrogels was measured in terms of the swollen-weight-to-dry-weight ratio.
Calculation of the average mesh size defining the linear distance between consecutive cross-linking points

The chemical cross-linked network structure is the most distinctive feature of the PEG-DA hydrogel. This network structure has been characterized in terms of $M_c$, the average molecular weight between cross-links, and the average mesh size, $\xi$. As shown in Figure S3, our calculations show that the average mesh size of PEGs with a molecular weight of 8k and with various wt% is less than 50 Å which is much smaller than the viral particles used to infect the encapsulated cells.

![Figure S3](image.png)

**Figure S3**
The average mesh size, $\xi$, (Å) of various PEG-DA hydrogels. $\xi$ was determined as described in equation 5.
Comparison between tensile stress and tensile strain tension for PEG-DA hydrogels of different molecular weights

Since the hydrogel is chemically cross-linked to form a network, it is important to test the sustainability of mechanical properties for different molecular weights of the PEG-DA hydrogel system. In addition, we need to confirm the integrity of the network while performing the experiments since we are attempting to diffuse the viral particles over the time course of our viral infection experiments. In order to monitor the structural integrity of the hydrogel networks, we employed uniaxial strip extensometry to measure the stress and strain of different MWs of poly(ethylene glycol) diacrylate over the experimental period. For PEG-DA mechanical testing, we used uncured solution loaded between glass slides (VWR) with a Teflon spacer and exposed to 320–390 nm UV light (10 mW/cm²) for 90 sec and P.I. of ~ 0.5 %. Figure S4-1 demonstrates the typical stress-strain curve of different molecular weights PEG-DA single-network hydrogels under elongation. Low molecular weight gels (PEG-DA 575 and 700) break at stresses of less than 10 kpa. As expected, higher molecular weight gels (PEG-DA 8k) tolerate up to 40 kpa, as we expected. Moreover, it is interesting that PEG-DA 8k retained its mechanical characteristics (typical stress-strain relationship), after up to ten days of swelling in the buffer, as shown in Figure S4-2. Using the modulus that calculates the tensile stress at maximum load divided by tensile strain at maximum load, we summarized the relationship between the modulus and the average effective mesh size. The data clearly shows that as the average mesh size decreases, the modulus increases.
Figure S4-1

Tensile stress versus tensile strain for PEG-DA hydrogels of various molecular weights.
Figure S4-2
Tensile stress versus tensile strain for aging PEG-DA 8k hydrogels.
Figure S4-3
Modulus versus mesh size for PEG-DA hydrogels of various molecular weights.