# Hydrolytically Degradable Microgels for Immune Cell Encapsulation

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**Statement of Purpose:** Micron-scale hydrogel particles (microgels) are attractive cell delivery platforms engineered to provide biological and physical support to enhance cell engraftment and sustain long-term release of paracrine molecules, enabling enhanced therapeutic actions. Microfluidics-based methods have arisen as a powerful technology for the high-throughput generation of microgels of controlled size, providing a rapid and reproducible methodology for cell encapsulation. Here, we report hydrolytically degradable microgels with tunable degradation kinetics using ethylene glycol bis-mercaptoacetate (EGBMA) as hydrolytic linker, which can be used as cell delivery platforms. Thus, we avoid the use sequence-specific enzymatic degradation of peptide, whose type and concentration can change spatiotemporally at the *in vivo* delivery site.

**Methods:** Microgels were fabricated in a microfluidic water-in-oil droplet generator as described[1]. Microgels were crosslinked with a solution containing dithiothreitol (DTT), or a mixture of DTT and EGBMA at different molar ratios (1 and 2 mM), for non-degradable and degradable microgels, respectively. Ly3 cells ($20x10^6$ cells/mL) were resuspended in 5% w/v PEG-4MAL polymer solution, which was pre-functionalized with a 1kDa SH-PEG-FITC for *in vitro* tracking (Figure 1a). Microgel degradation was monitored by FITC release over time and optical microscopy. Cell viability was evaluated by Live/Dead assay using confocal imaging.

**Results:** Hydrolytic degradation of microgels results in PEG-FITC linker cleavage from the PEG-4MAL backbone. Thus, microgel degradation was evaluated by tracking the fluorescence intensity of PEG-FITC linker presence in the solution of microgels at 37°C in dPBS over time (Figure 1b). The higher fluorescence intensity was found for 2 mM degradable microgel group ($p<0.0001$ vs DTT and 1 mM degradable microgel group from 1 to 12 days of culture), followed by 1 mM degradable microgels group ($p<0.001$ vs DTT from 3 to 12 days of culture, $p<0.001$ vs 2 mM from 1 to 12 days of culture). Ly3 cells were successfully encapsulated in the degradable microgels, showing homogeneous distribution (Figure 1c). Degradation of encapsulated cells microgel was also monitored by optical microscopy over time. Brightfield images in Figure 1c show a faster degradation for 2 mM degradable microgels, followed by 1 mM degradable microgels in comparison to DTT microgels. By 3 days of culture, 1 mM degradable microgels showed diffuse microgel outlines, indicating some degradation in comparison to DTT microgels, whose outlines are clearly visible. For 2 mM degradable microgels group, the microgels were completely degraded and the encapsulated cells were released after 3 days of culture. These results indicated that the degradation kinetics of the microgels, and consequently cell release, can be easily tuned over time by changing the molar ratio of EGBMA linker in the polymer fraction. Viability of encapsulated cells in 1 mM degradable microgels was evaluated by live/dead assay and confocal microscopy (Figure 1e and 1f). Encapsulated Ly3 cells showed 65% cell viability after encapsulation, which increased up to 70% ($p<0.05$) after 2 days of culture.

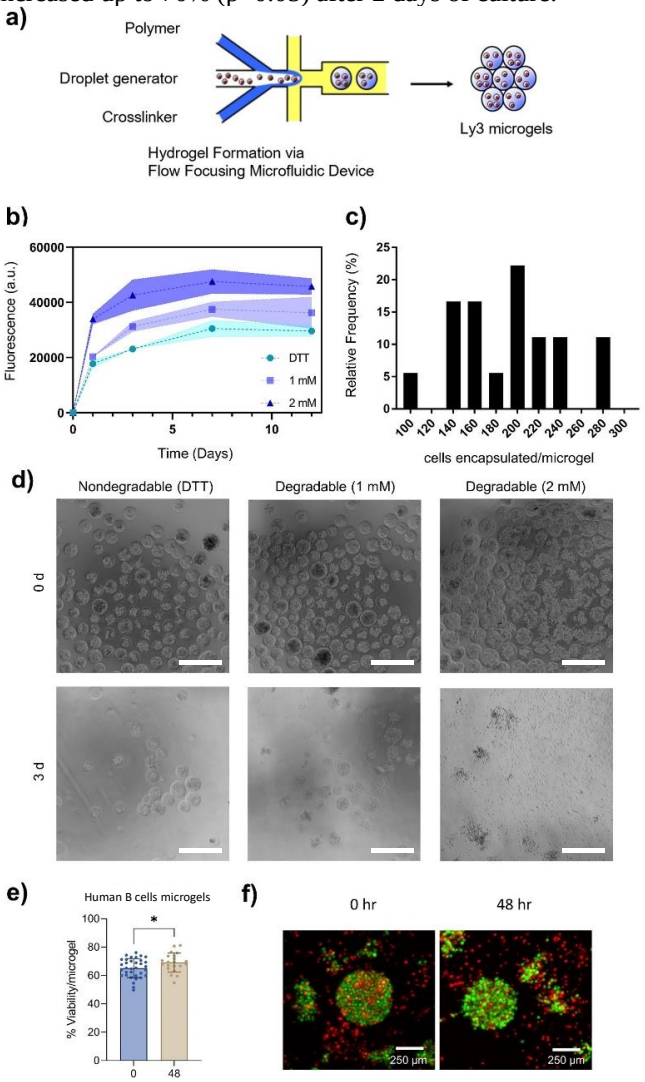


**Figure 1: a)** Microgel forming flow-focusing device**; b)** FITC release from PEG-4MAL microgels over time in dPBS at 37 ºC; **c)** Encapsulated Ly3 cell distribution in PEG-4MAL microgels; **d)** Optical microscopy images of encapsulated Ly3 cells in PEG-4MAL microgels after fabrication and 3 days of culture. Scale bar: 500 µm; **e)** Viability of encapsulated Ly3 cells in 1 mM degradable microgels after synthesis and 48 h of culture; **f)** Live/Dead assay of encapsulated Ly3 cells in 1 mM degradable microgels.

**Conclusions:** We demonstrated that cells can be encapsulated in monodisperse hydrolytically degradable microgels through flow-focusing microfluidics. The combination of a hydrolytic ethylene linker along with a non-degradable thiol linker provides tunable microgel degradation kinetics and therefore, tunable *in vitro* cell release.

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**References:** [1] Headen et al. Microsystems & Nanoengineering 4.1 (2018). 1-9.