

Supplemental Material

Bacteria Encapsulation into Polyethylene Glycol Hydrogels Using Michael-Type Addition Reactions

Moises M. Gutierrez¹, Jeffrey A. Reed¹, Robby A. McElroy¹, Ryan R. Hansen¹

¹ Tim Taylor Department of Chemical Engineering, Kansas State University, Manhattan, KS, United States.

Materials

Bacillus subtilis was obtained from ATCC (ATCC 6051) and *B. subtilis* expressing GFP (*B. subtilis*-GFP), was obtained from the Bacillus Genetic Stock Center (Strain 1A1135). Both strains were maintained in a 50% stock glycol solution at -80°C. PEGDA, PEGVS, and PEGMA were obtained from Creative PEGworks (PSB-347, PSB-3223, and PSB-301), and PEGTT from NOF America (SUNBRIGHT PTE-100SH).

Live-Dead assay calibration curve

The calibration curve was prepared following the live/dead assay kit instructions manual [1]. *Bacillus subtilis* subsp. *subtilis* (ATCC 6051) was used in all live-dead experiments. To generate the calibration curve, four cell suspensions were used, representing 0%, 33%, 67%, and 100% of living cells in solution. Standards were made by preparing two 600 μ L solutions with cells suspended with an OD₆₀₀ index of 0.1. The first solution contained 0% live cells, and the second contained 100% live cells. For this first solution, *B. subtilis* cells were killed by incubating 70% isopropanol at room temperature for 20 minutes, then centrifuged and resuspended in water. The other two standards for the calibration curve points were made using a combination of the 0% and 100% live cell suspensions. To prepare the 67% standard, 100 μ L of the 0% live cell suspension was mixed with 200 μ L of the resuspended 100% live cell solution. Similarly, the 33% standard was made by mixing 200 μ L of the 0% live cell suspension with 100 μ L of the 100 % live cell solution. Syto 9 was used to label intact live and compromised dead cells, while propidium iodide was used to label the compromised cells. Green and red images of cell populations were measured on a Nikon Ti-U inverted microscope with a 10X, NA 0.3 objective using FITC and TRITC filters.

Hydrogel Encapsulation

For encapsulation, 12.5 μ L of the bacteria solution was first mixed with 5.6 μ L of PEGDA or PEGVS, then 6.9 μ L PEGTT was added, initiating hydrogel formation. 7 μ L of the mixed precursor solution was then quickly pipetted between an 18x18 mm thiolated glass coverslip and a fluorinated (inert) glass coverslip separated by two 12.5 μ m spacers. Solutions were incubated for 25 min, at which point the inert coverslip was removed, leaving the resulting hydrogel covalently bonded to the thiolated coverslip. Due to the rapid reaction kinetics of the thiol-maleimide reaction, the PEGMA and PEGTT hydrogel precursors were not premixed but were instead mixed directly between the two glass coverslips at 7 μ L volumes.

Hydrogel Mesh Size

Average hydrogel mesh size for each hydrogel was estimated by measuring dry and swollen hydrogel mass, as described by Canal and Peppas [2]. For both the swollen and dry hydrogel mass, three 200 μL hydrogels were formed in a 2 mL glass jar. For swelling, 500 μL of water was pipetted on top of the gel and allowed to equilibrium; for the dry mass, the 200 μL hydrogels were placed in an oven at 80°C for 24 hours. All constants required by the Canal and Peppas' method for calculating the mesh size are listed (Table S1).

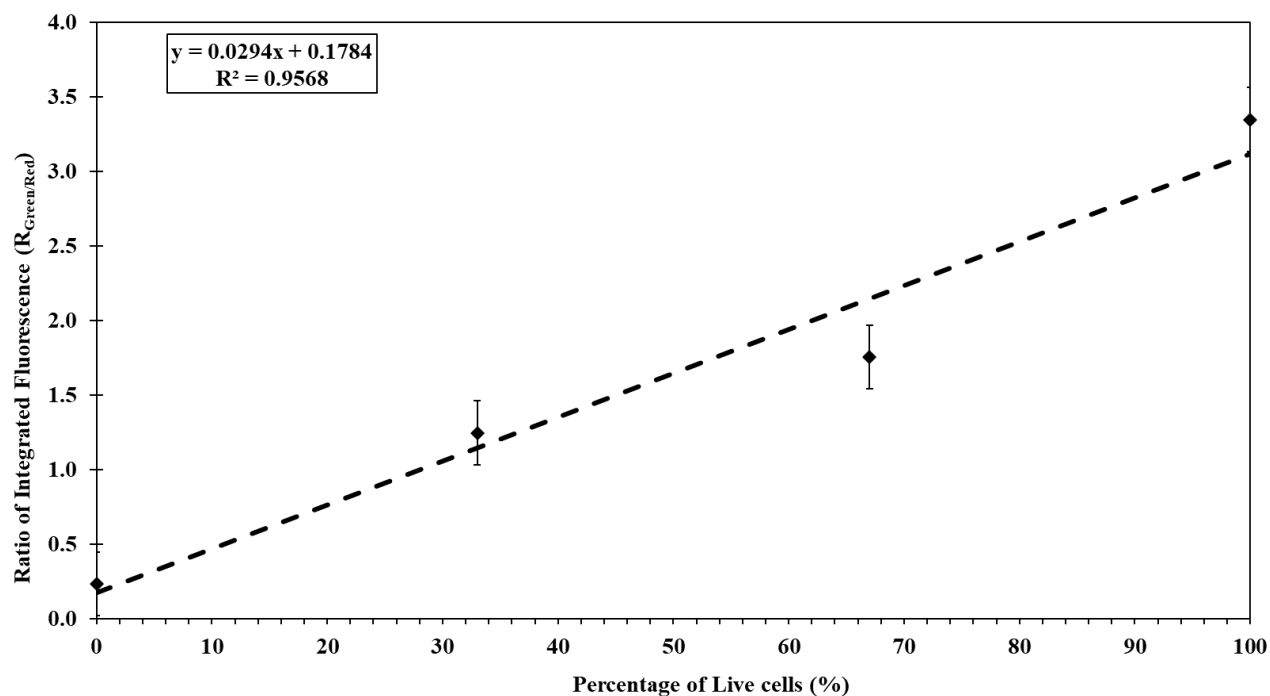


Fig S1. Calibration curve for Live/Dead assay.

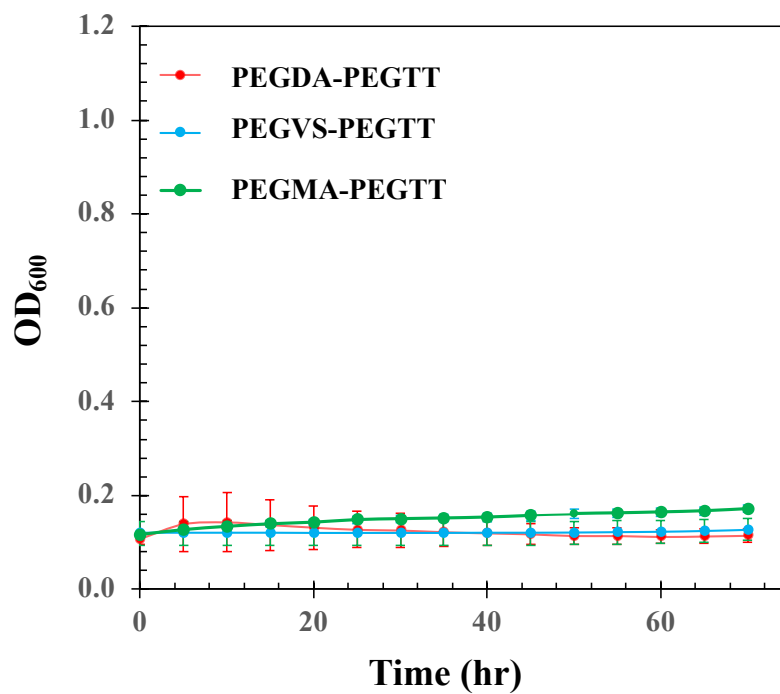


Fig. S2. Growth profiles for empty hydrogel control (no bacteria cells) during incubation in ATGN growth media ($n \geq 3$).

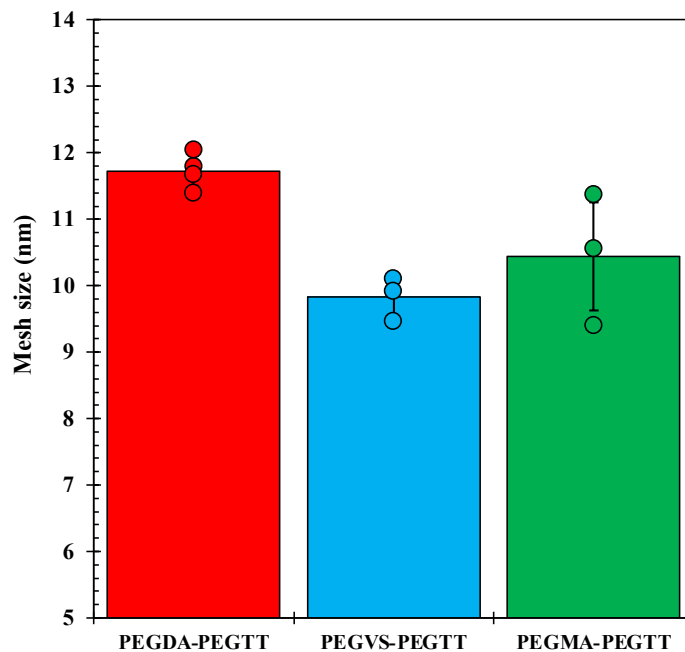


Fig. S3. Calculated mesh sizes for each hydrogel ($n \geq 3$, $*p < 0.05$). Results are reported as mean $\pm$ standard deviation.

Table S1. Constant used to calculate hydrogel average mesh size using equilibrium swelling theory [2]

Constant	Symbol	Value	Reference
Average bond length (nm)	l	0.146	[3]
Molecular weight of the repeat unit (g/mol)	M_r	44.0	
Molar volume of the solvent (cm ³ /mol)	V	18.0	
Polymer-solvent (PEG-water) interaction parameter	χ	0.426	[3-4]
Flory characteristic ratio of the polymer	C_n	4.0	[5]
The number-average molecular weight of each monomer between crosslink points *	M_n	8400	
Density of polymer (g/cm ³)	ρ_p	1.125	
Density of solvent (g/cm ³)	ρ_s	1.000	
The specific volume of the polymer (ρ_s/ρ_p)	v	0.889	

*3,400 kDa for the 2-arm monomer and two 2,500 kDa for the 4-arm monomer

References

1. Thermo-Fisher Scientific. LIVE/DEAD™ BacLight™ Bacterial Viability Kits. Revised 15-July-2004.
2. T. Canal, N. A. Peppas. Correlation between Mesh Size and Equilibrium Degree of Swelling of Polymeric Networks. *J. Biomed. Mater. Res.* 1989, **23** (10), 1183–1193. <https://doi.org/10.1002/jbm.820231007>.
3. S. P. Zustiak, J. B. Leach. Hydrolytically Degradable Poly(Ethylene Glycol) Hydrogel Scaffolds with Tunable Degradation and Mechanical Properties. *Biomacromolecules* 2010, **11** (5), 1348–1357. <https://doi.org/10.1021/bm100137q>.
4. G. M. Cruise, D. S. Scharp, J. A. Hubbell. Characterization of Permeability and Network Structure of Interfacially Photopolymerized Poly(Ethylene Glycol) Diacrylate Hydrogels. *Biomaterials* 1998, **19** (14), 1287–1294. [https://doi.org/10.1016/S0142-9612\(98\)00025-8](https://doi.org/10.1016/S0142-9612(98)00025-8).
5. M. S. Rehmann, K. M. Skeens, P. M. Kharkar, E. M. Ford, E. Maverakis, K. H. Lee, A. M. Kloxin. Tuning and Predicting Mesh Size and Protein Release from Step Growth Hydrogels. *Biomacromolecules* 2017, **18** (10), 3131–3142. <https://doi.org/10.1021/acs.biomac.7b00781>.