Hydrogel synthesis via β-thiolactone-mediated native chemical ligation for cell encapsulation

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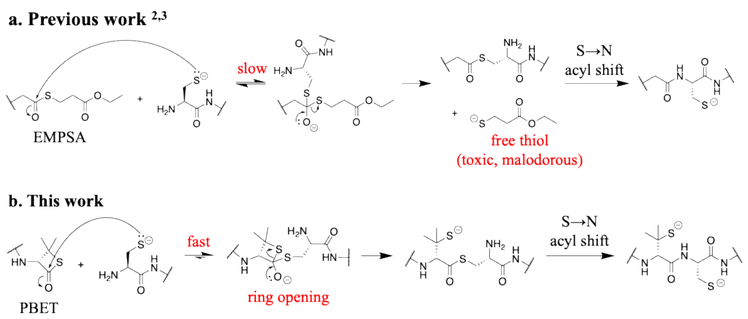
Introduction

3D cell culture advancements are continuously improving upon cross-linking strategies for hydrogel cell encapsulation. Researchers still face challenges with creating rapid, nontoxic, inexpensive, and spontaneous methods for hydrogel cell encapsulation.[1,2] Native chemical ligation (NCL) is a promising method for hydrogel formation to link polyethylene glycol (PEG) scaffolds but suffers two major drawbacks. Specifically, the reaction produces a cytotoxic free thiol byproduct and exhibits slow kinetics, both due to the alkyl thioester of ethyl 3-mercaptopropionate-succinic acid (EMPSA).[3,4]



Scheme 1. *Limitations of previous work compared to this work.*
a. The reaction scheme of 4-arm PEG-EMPSA with 4-arm PEG N-terminal cysteine (cys) revealing slow kinetics and cytotoxic free thiol.
b. Our proposed 4-arm PEG-PBET with 4-arm PEG-cys displays faster kinetics through the β-thiolactone ring opening while retaining the thiol leaving group within the PEG backbone.

We proposed a method for 3D cell encapsulation in PEG hydrogels through β-thiolactone mediated NCL. We can form networks with optimal gelation kinetics while avoiding cytotoxic free thiol byproducts. This was achieved by functionalizing 4-arm PEG with a cyclized thioester derived from the amino acid penicillamine: penicillamine-β-thiolactone (PBET).

Experimental Design

NCL PEG Hydrogel with β-thiolactone allowing for <2 min gelation time and elimination of cytotoxic free thiols

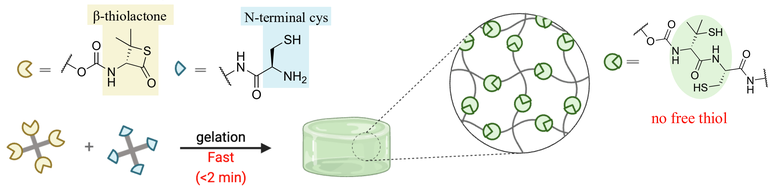


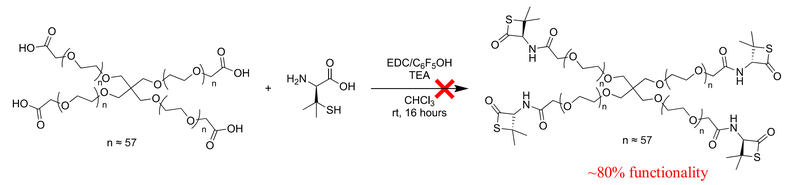
Figure 1. *Fast gelation of 4-arm PEG-PBET and 4-arm PEG-cys without toxic free thiol byproduct. Resulting thiol handles on the PEG backbone provide a functional handle for post-gelation functionality and tunability."*

References

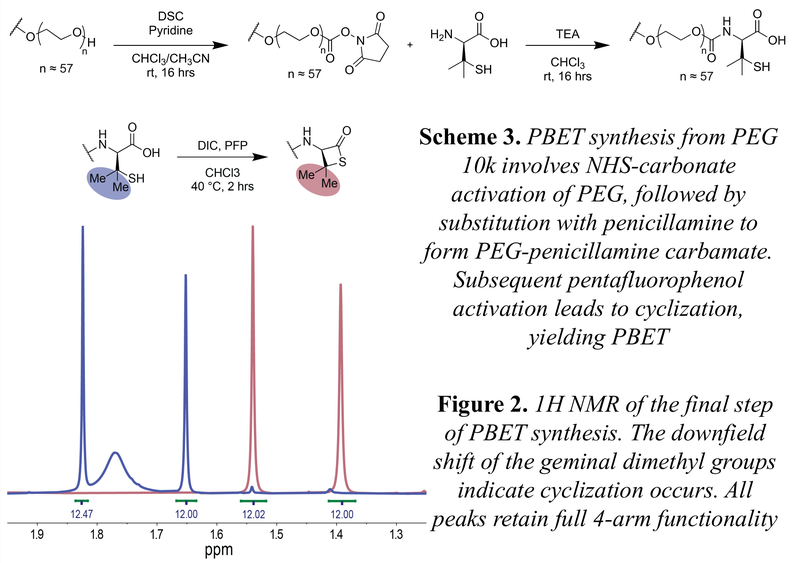
1. Echalier, C.,et al., E. S. *Mater. Today Commun.* **2019**, *20*, 100536.
2. Chen, H., et al., *Chem. Sci*. **2018**, *9* (7), 1982–1988.
3. Hu, B.H., et al., *Biomacromolecules* **2009**, *10* (8), 2194–2200.
4. Jung, J.P., et al., *Biomacromolecules* **2013**, *14* (9), 3102–3111.

Synthesis of PBET

We attempted a synthesis route with addition and cyclization in one step. Functionality was lost in the work-up step. After multiple different isolation attempts, carbamate synthesis was found to be the optimal route of addition and cyclization, leading to the three-step synthesis now used for PBET.



Scheme 2. *Example of unsuccessful PBET synthesis. Jones oxidation afforded PEG Acid 10k, but various subsequent coupling reactions could not achieve full functionality of the PBET product*



Scheme 3. *PBET synthesis from PEG 10k involves NHS-carbonate activation of PEG, followed by substitution with penicillamine to form PEG-penicillamine carbamate. Subsequent pentafluorophenol activation leads to cyclization, yielding PBET*

Figure 2. *1H NMR of the final step of PBET synthesis. The downfield shift of the geminal dimethyl groups indicate cyclization occurs. All peaks retain full 4-arm functionality*

Cell Viability

Cell viability to study if PBET NCL gel formation removed the free thiol toxicity as hypothesized. Exogenous free thiols lead to protein misfolding and negatively impacts glutathione metabolism. Live-dead assays were monitored with laser scanning confocal microscopy (LSCM).

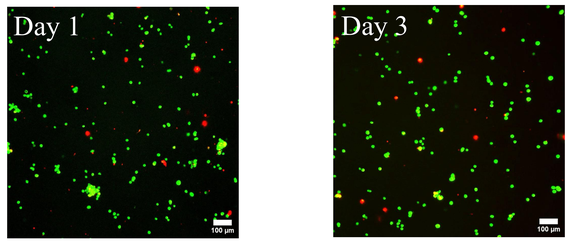
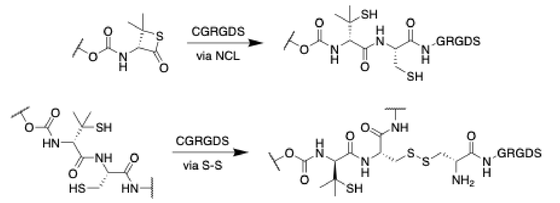


Figure 3. *LSCM images of 3D cell culture of human dermal fibroblasts (HDF). 5 wt% gel, 4 mM RGD. Live-dead assay of days 1 and 3. CalcienAM (live, green) and Ethidium homodimer-1 (dead, red)*

Cell Adhesion



Scheme 4. *Attachment methods of RGD adhesion peptides for spontaneous incorporation into gel via NCL at gelation or disulfide bridges post-gelation.*

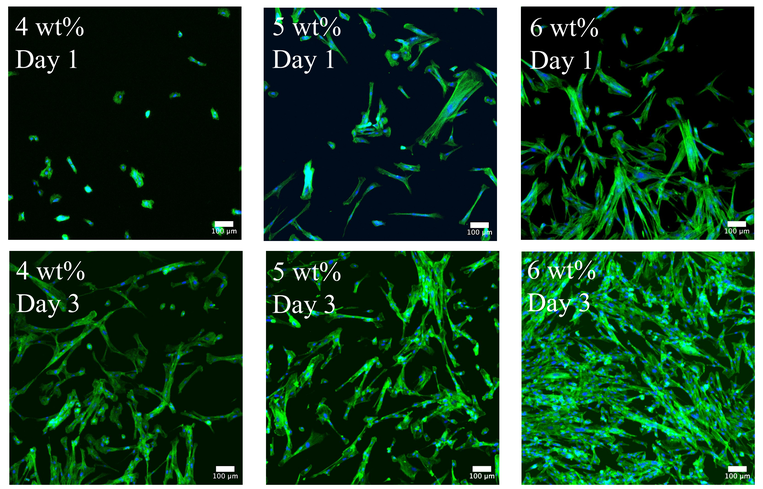


Figure 4. *2D cell studies with HDF. LSCM images show increased PEG content promotes 2D growth. Day 1 and day 3, 4 mM RGD. DAPI (DNA, blue), Phalloidin 488 (actin, green)*

Ongoing Work

The rapid gelation of our system has shown potential for fiber pulling. We are currently exploring this with preliminary cell studies show promising results.

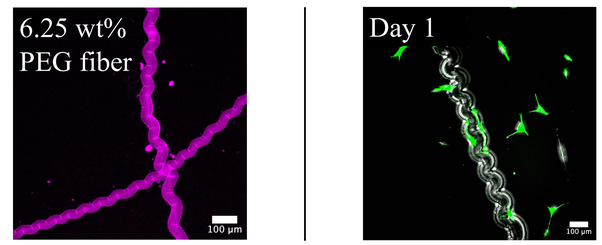


Figure 5. *Fiber adhesion cell studies with HDF.*
(R) PEG Fiber, 6.25 wt% stained with Nile Red.
(L) LSCM show cell growth across and within fiber grooves. 12.5 wt% fibers.
DAPI (DNA, blue), Phalloidin 488 (actin, green)

Acknowledgments

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