Tunable amphiphilic diblock copolymers with enhanced drug loading capacity and their toxicity evaluation through microfluidics

Himanshu Polara, Hanghang Wang, Tejas V. Shah, Godwin Babanyinah, Abhi Bhadran, Michael C. Biewer, Mihaela C. Stefan*

Department of Chemistry and Biochemistry, The University of Texas at Dallas, Richardson, TX 75080. E-mail: Himanshu.Polara@utdallas.edu

Cancer

- Expected to be the leading cause of death in every country in the 21st century.
- There are 18.1 million new cancer cases and 9.6 million cancer deaths worldwide in 2018.

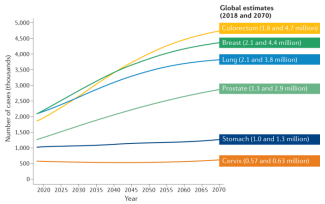


Figure 1. Projected worldwide cancer cases from 2020–2070.[1]

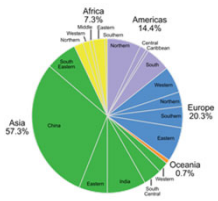
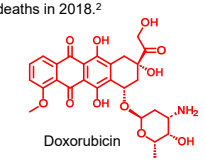


Figure 2. Distribution of worldwide cancer deaths in 2018.[2]

Limitation of chemotherapy:

- Commonly used doxorubicin (DOX) is poorly water-soluble
- Poor solubility in the bloodstream
- Harmful side effects, such as DOX-induced cardiotoxicity.



Polymeric Micelle for Drug Delivery

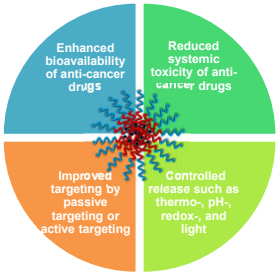


Figure 3. Advantages of drug delivery by polymeric micelle carriers.

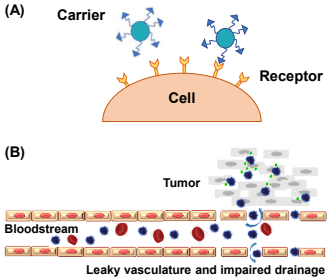


Figure 4. (A) Active targeting and (B) passive targeting.

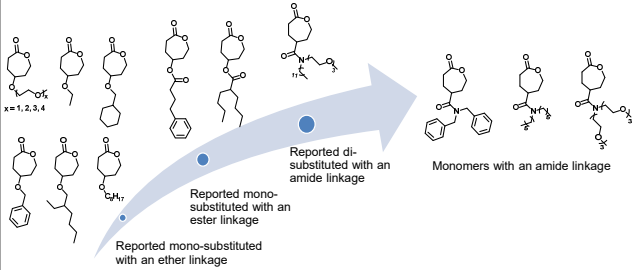


Figure 5. The development of γ-functional ε-caprolactone monomers.[3, 4]

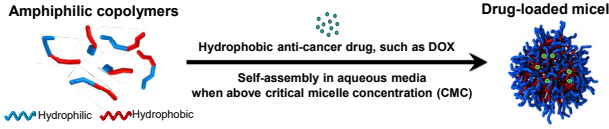
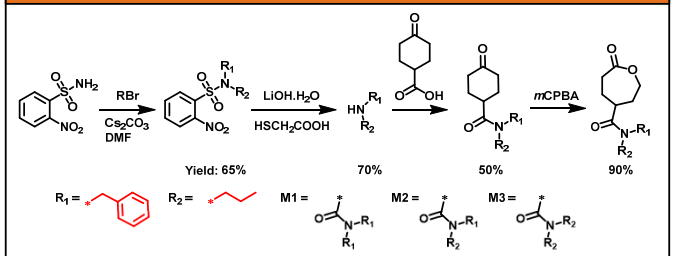


Figure 6. Self-assembly of amphiphilic copolymer to form drug-loaded polymeric micelles.

Synthesis and Characterization



Scheme 1. Synthesis of disubstituted γ-amide functionalized caprolactones (ε-CL).

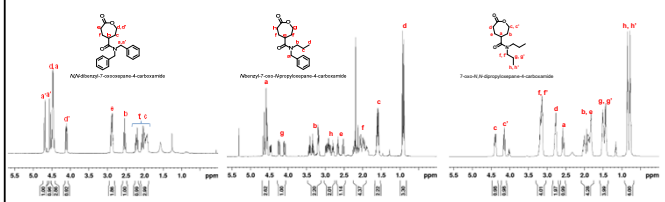
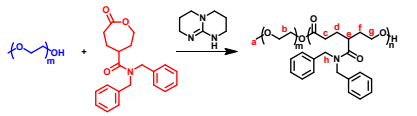


Figure 7. ^{1}H NMR of synthesized hydrophobic monomers; Bn_2CL, BnPyCL, Py_2CL (left to right).



Scheme 2. Ring-opening polymerization of ε-CL monomer using poly(ethylene glycol) (PEG) as an initiator and 1,5,7-triazabicyclo[4.4.0]dec-5-ene as a catalyst.

Table 1. Summary of molecular weights, compositions of synthesized block copolymers, and their relative drug loading capacities.

Polymer	mol% Hydrophilic : Hydrophobic	M_n g/mol (NMR)	M_n g/mol (SEC)	PDI (SEC)	CMC (g/L)	Size (dispersity) (nm) Empty	Size (dispersity) (nm) DOX-loaded	Ratio Polymer : DOX	DOX loading capacity (wt%)
PEG-*b*-PBn$_2$CL	49:51	18,550	6,300	1.3	1.16 x 10^{-3}	120.2 (0.129)	134.0 (0.149)	10:1	5.55
PEG-*b*-PBnPyCL	52:48	16,600	4,600	1.9	1.50 x 10^{-4}	121.0 (0.105)	137.9 (0.164)	10:1	7.33
PEG-*b*-PPy$_2$CL	53:47	7,900	4,200	1.1	6.00 x 10^{-4}	83.40 (0.131)	139.6 (0.091)	10:1	3.70

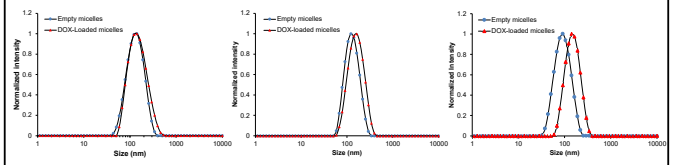


Figure 8. Hydrodynamic sizes of empty and DOX-loaded micelles of PEG-*b*-PBn$_2$CL, PEG-*b*-PBnPyCL, PEG-*b*-PPy$_2$CL (left to right).

In-vitro drug Release

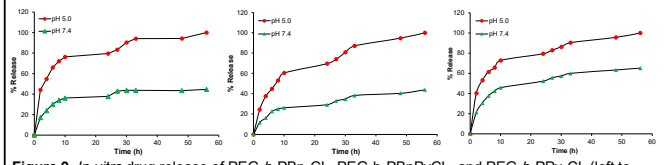


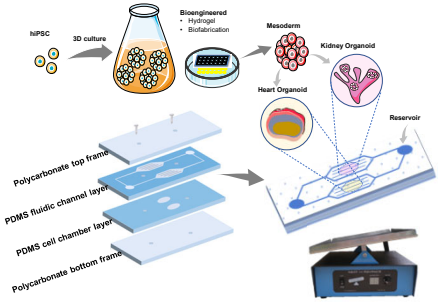
Figure 9. *In-vitro* drug release of PEG-*b*-PBn$_2$CL, PEG-b-PBnPyCL, and PEG-*b*-PPy$_2$CL (left to right) at acidic and physiological pH.

Future Directions

❖ **Combination loading of DOX with natural antioxidant resveratrol (RSV)[5]**

Polymer	[Polymer]:[RSV]:[DOX]	DLC^{RSV} (%)	EE^{RSV} (%)	DLC^{DOX} (%)	EE^{DOX} (%)
PEG-*b*-PCL	10:1:0	0.24	2.4	-	-
	10:0:1	-	-	0.96	9.6
	10:1:1	0.06	0.6	1.08	10.8
PEG-*b*-PBCL	10:1:0	0.22	2.2	-	-
	10:0:1	-	-	3.10	31.0
	10:1:1	1.87	18.7	8.77	87.7

❖ **Pumpless device[6] with gravity-induced flow to mimic physiological conditions for culturing heart and kidney organoid**



- Easy set-up and operation
- Cost effective
- No bubbles
- Realistic shear stress

Summary

Statement of Challenge

- ❑ We are designing and synthesizing various γ-functionalized ε-caprolactones used for the ring opening polymerization to generate amphiphilic diblock copolymers for micellar drug delivery.
- ❑ The biological studies include 2D and 3D cell culture in microfluidic devices. We designed and fabricated heart-liver-on-chip microfluidic devices and plan to advance the fabrication of heart-kidney organoid-on-a-chip microfluidic devices.

Summary of Key Elements and Finding of Research

Novel γ-functionalized ε-caprolactone monomers to generate amphiphilic diblock copolymers with tunable composition, molecular weight, size, and drug loading capacity of micelles. Co-loading with polyphenols resulted in increased drug loading capacity.

Potential for Positive Impact

The synthetic platform developed for caprolactone monomers can be modified to synthesize amide-functionalized caprolactone monomers. This can result in polymers with high drug loading capacity and better cellular uptake. A multiorganoid-on-chip model will be used to predict the toxicity of the loaded micelles.

References

1) Soerjomataram, I.; Bray, F. Planning for tomorrow: global cancer incidence and the role of prevention 2020–2070. *Nat Rev Clin Oncol* **2021**,18, 663–672.
2) Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R. L.; Torre, L. A.; Jemal, *CA: a cancer journal for clinicians* **2018**, *68*, 394–424.
3) Calubaquib, E.L.; Soltantabar, P.; Wang, H.; Shin, H.; Flores, A.; Biewer, M.C.; Stefan, M.C. *Polymer Chemistry* **2021**, 12(24), 3544-3550.
4) Bhadran, A.; Shah, T.; Babanyinah, G.K.; Polara, H.; Taslimy, S.; Biewer, M.C.; Stefan, M.C. Recent Advances in Polycaprolactones for Anticancer Drug Delivery. *Pharmaceutics* **2023**, 15, 1977.
5) Washington, K.E.; Kularatne, R.N.; Biewer, M.C.; Stefan, M.C. *ACS Biomaterials Science & Engineering* **2018**, *4*(3), 997-1004.
6) Soltantabar, P.; Calubaquib, E.L.; Mostafavi, E.; Ghazavi, A.; Stefan, M.C. *Organs-on-a-Chip* **2021**, *3*, 100008.

Acknowledgements

❑ **GRaCE Fellowship – Office of Research and Innovation, UTD**
❑ **Robert A. Welch Foundation – AT1740**
❑ **National Science Foundation – NSF CHE-1566059 & CHE-1609880**