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Nanoparticle Rigidity Influences the Uptake by Human Glioblastoma Cells

Chung-Fan Kuo, Fereshtehsadat Mirab , and Sheereen Majd
Department of Biomedical Engineering, University of Houston

Background and motivation

- Intrinsic properties of nanoparticles (NPs) are known as strong determinants of NP's biological interactions during the delivery.
- NP rigidity has shown an influence on particles' association with and internalization into few different sources of the tumor cells.
- The role of NP rigidity on brain glioma tumor cells has not been studied and may present an opportunity to improve the NP-based treatments for brain cancer.

Goal

- This study aims to investigate the importance of NP rigidity for its interactions with glioma cells *in vitro.*

Experimental approach

1. Examined the rigidity of poly(ethylene glycol) diacrylate (PEGDA) (Fig. 1A) in bulk form using compression test by a rheometer (TA Instruments AR 2000EX) (Fig. 1B) to determine their elastic moduli with different PEGDA volume ratios. These gel solutions contained a concentration of 1 v/v% photo-initiator.

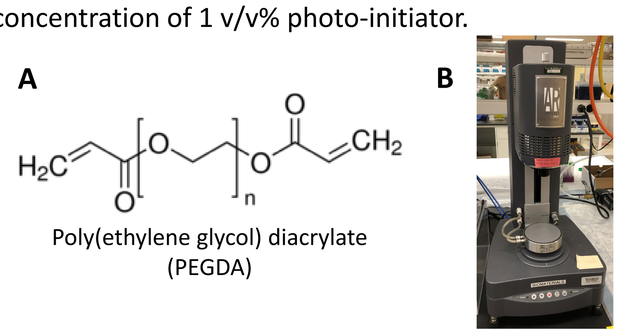
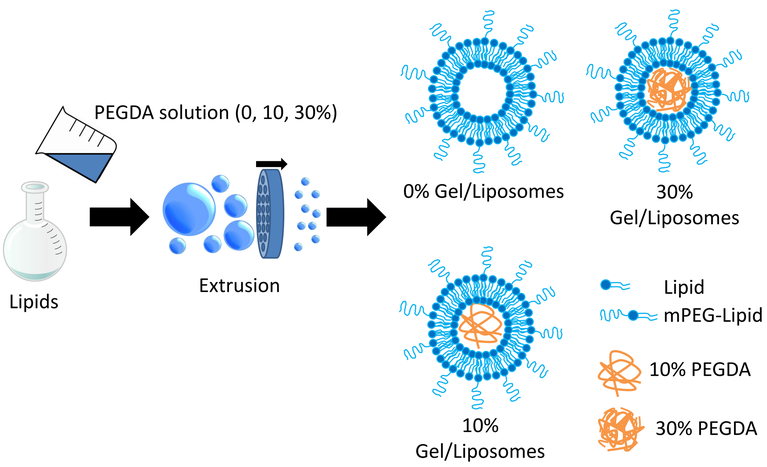


Figure 1. (A) Chemical structure of PEGDA. (B) AR2000EX Rheometer.

2. Using a simple extrusion method to encapsulate different percentages of PEGDA (0, 10, and 30%) within nano-liposome's lumen to vary rigidity levels.



3. The size distribution and zeta potential of gel/liposomes were measured using a Zetasizer (Malvern Panalytical).
4. Qualitative analysis and quantitative analysis of NP uptake by human glioblastoma cells (U87) were carried out by confocal imaging and flow cytometry (FACS analysis), respectively.

Results: Elasticity of PEGDA bulk gels

- The elastic modulus of PEGDA gel with increasing volume percentage (Fig. 2A) increased significantly, from 0.1 to 8 MPa (Fig. 2B).

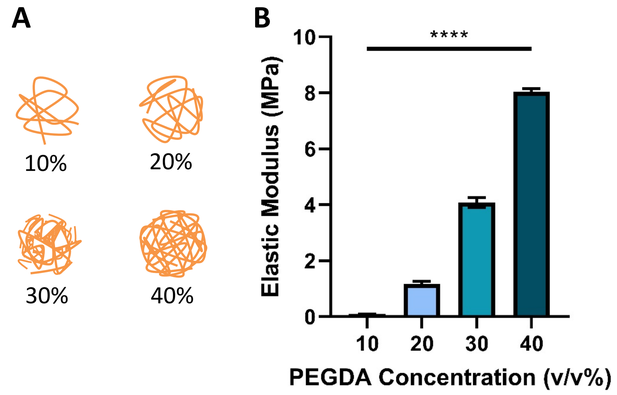


Figure 2. (A) Schematic of different PEGDA volume ratios. (B) Elastic modulus of different ratio of PEGDA bulk gels. n=5, ****< 0.0001

PEGDA percentage	10%	20%	30%	40%
Elastic modulus (MPa)	0.27 ± 0.37	1.17 ± 0.10	4.08 ± 0.18	8.05 ± 0.11

Results: Size distribution and zeta potential of gel/liposomes

- Inclusion of PEGDA hydrogel in liposomal lumen did not have an impact on their size distribution (Fig. 3A), showing a similar particle size within 114 to 119 nm with polydispersity index (PDI) <0.14 based on dynamic light scattering data.
- All these liposomes had similar zeta potential (-1.9 to -2.7 mV) further confirming their surface similarities (Fig. 3B).

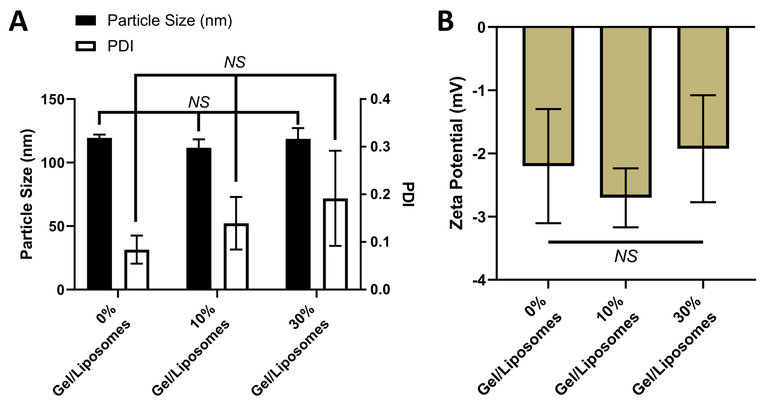


Figure 3. Particle size and PDI (A), and Zeta potential (B) of 0, 10, and 30% of gel/liposomes. n=3, *NS*: non-significant

Gel/Liposomes	0%	10%	30%
Size (nm)	119.47 ± 2.64	111.73 ± 6.60	118.73 ± 8.46
PDI	0.08 ± 0.03	0.14 ± 0.06	0.19 ± 0.10
Zeta potential (mV)	-2.20 ± 0.90	-2.70 ± 0.47	-1.93 ± 0.85

Acknowledgement

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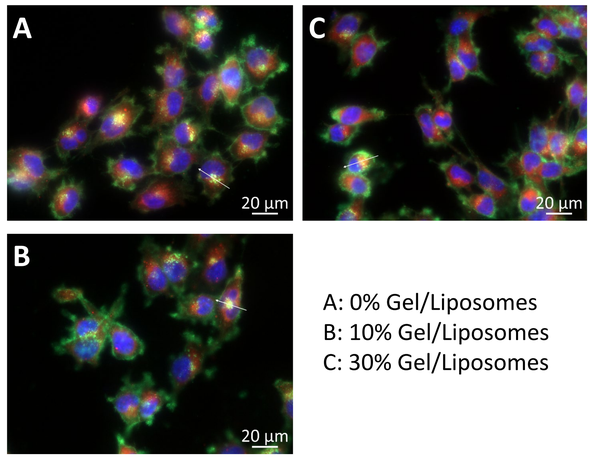
Results: Confocal imaging of U87 uptake

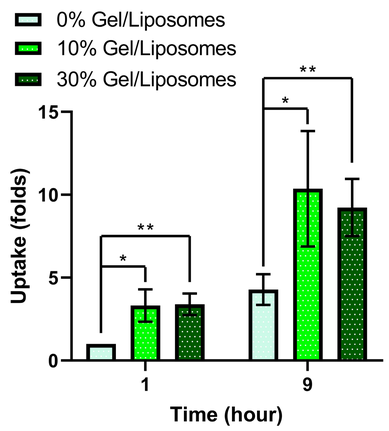
- All NPs were incubated with U87 cells for 6 hours at 37°C.
- Fluorescence images were taken using a confocal microscope (LSM800) with a 63x oil lens.
- All NPs were seen accumulating inside the cytoplasm of U87 cells.



A: 0% Gel/Liposomes
B: 10% Gel/Liposomes
C: 30% Gel/Liposomes

Results: FACS analysis of U87 uptake

- No differences between liposomes with 10% and 30% gel core after 1- and 9-hour incubation periods were found.
- The liposomes with a PEGDA hydrogel core presented a significantly higher uptake compared to the liposomes without a PEGDA core.
- Liposomes with 10% and 30% gel cores had about 2.5 and 2 times higher uptake by U87 compared to liposomes with 0% gel at 9 hours.
- This result suggests that U87 are more partial to uptake NPs with a supporting structural core than their much softer counterparts.



Gel/Liposomes	0%	10%	30%
1 hour	1.00 ± 0.00	3.32 ± 0.97	3.39 ± 0.65
9 hours	4.28 ± 0.93	10.36 ± 3.48	9.23 ± 1.73

Conclusions

- All NPs had a similar particle size distribution (mean ~117 nm, PDI <0.14), and zeta potential (~ -2.3 mV).
- FACS data suggested harder NPs are more favorable by U87 than soft ones.
- Confocal imaging confirmed that these liposomes were up-taken by U87 cells where NPs were seen at the cytoplasm.