Polybubble Depots Functioning as a Theranostic-enabling Anti-cancer Platform Technology

Shreedevi Arun Kumar, Jacob Good, David Hendrix, Whitney N. Souery, Yong-Yu Jhan, Corey J. Bishop
Department of Biomedical Engineering, Texas A&M University

Statement of Significance: Combinatorial therapies are often used to treat various cancers to combat the development of drug resistance. However, drug toxicity is a major challenge in administering the small molecules, thereby limiting the applications. There is a need for drug delivery platforms that have the potential to optimize drug dosage by controlling release kinetics of the synergistic small molecules. This platform can also be potentially used for single injection vaccines to improve vaccine coverage in developing countries. We have developed a theranostic-enabling, polyester-based polybubble drug delivery platform that can enable novel release profiles, such as delayed bursts. To further enable on-demand control of release extracorporeally and to track the polybubbles in an *in vivo* context, theranostic gold nanorods (AuNR)s are encapsulated within the shell of the polybubble.

Methods: Cetrimonium bromide (CTAB)-stabilized aqueous AuNRs were synthesized using sodium borohydride, silver nitrate, chloroauric acid, salicylic acid, and CTAB. The aqueous AuNRs were then hydrophobicized via thiolation using mPEG thiol and then were transferred to chloroform (organic phase). Non-acrylated PCL was purchased commercially and acrylate-terminated PCL was synthesized using PCL triol, acryloyl chloride, and potassium bicarbonate. PCL was dissolved in chloroform and mixed with 10% (v/v) AuNRs ($3.5x10^{12}$ particles/mL) and then was injected into a glass vial containing 10% CMC for maintaining sphericity via phase separation resulting in polybubbles which were yet again injected with an aqueous pocket of cargo (i.e., acriflavine/doxorubicin (DOX); detection via fluorescence). Polybubbles were cross-linked via UV (302 nm) and lyophilized overnight to remove residual solvent. Release studies for polybubbles were conducted in PBS at 37℃, 50℃, 70℃, and 90℃. Polybubbles with centered cargo were characterized by cutting the polybubbles in half, followed by SEM, fluorescence microscopy, and confocal microscopy. Polybubbles were heated using 801 nm near infra red (NIR) laser and the temperature difference was measured using FLIR images. Release studies were carried out for polybubbles that were periodically heated using the laser.

Results: The AuNRs' transverse and longitudinal diameters were 13 ± 4 nm and 50 ± 14 nm, respectively. Polybubbles with hydrophobicized AuNRs in the shell were heated using NIR laser, resulting in a temperature change of 10 ± 1 ℃. The SEM of the polybubbles showed the inner and outer morphology in the presence and absence of acriflavine. Drug centering was achieved in PCL polybubbles. Drug diffusion through the polymer shell was observed for three and half months to date (**Figure 1A1** and **1A2**). Release studies at 50℃ without laser activation showed a delayed burst release with centered cargo on day 62 (**Figure 1B**) (more studies are required to determine variability). Release studies at 50℃ with laser activation showed short bursts of cargo release (**Figure 1C**). Our polybubbles also appear to aid AuNRs by helping retain the AuNR shape, thus enabling the AuNRs to be reheated multiple times with a change of 12 ± 1℃ after laser activation (**Figure 1D**).

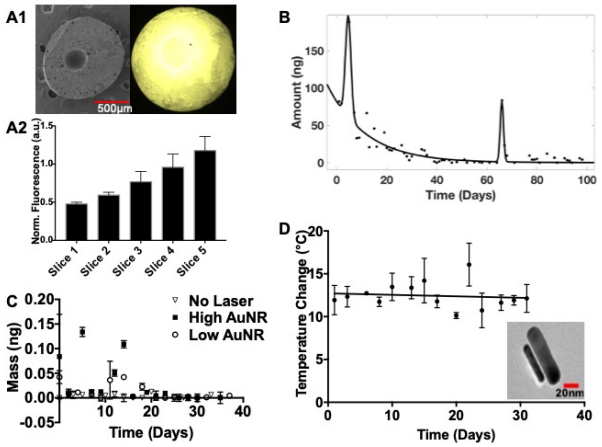

Figure 1. (A1) SEM and fluorescence microscope images of the polybubble and **(A2)** normalized fluorescence intensity for each slice of the polybubble from the surface of the polybubble to the border of the cargo; **(B)** release profile of the polybubble with cargo in the middle showing delayed burst release**; (C)** bursts of cargo release in polybubbles with AuNRs in the shell after each laser excitation (5 minutes); **(D)** Constant temperature change after multiple laser excitations of polybubbles with AuNRs in the shell.

Conclusion: We have successfully synthesized AuNRs with a surface plasmon resonance in the NIR (790 nm) region and we were able to phase transfer the AuNRs to an organic phase with high efficiency. We have demonstrated we are able to achieve a delayed burst effect using this polybubble platform and that we are able to cause controlled release based on the timing of the lasering. We were also able to demonstrate that our AuNRs are able to be heated without undergoing retraction after 1 month and 14 laser sessions. Currently there are on-going release studies for cargo in the shell (immediate continuous release) and cargo in the middle (delayed burst release) with and without laser activation. *In vivo* studies are on-going in a melanoma mouse model to determine the effectiveness of laser excitation of polybubbles *in vivo*. Future studies will entail modeling the pore sizes over space and time based on confocal images and predicting time of cargo release from the polybubbles.