Monitoring Controlled Release of Doxorubicin from Porous Microparticles using in vitro Models and Time Lapse Microscopy

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ABSTRACT SUMMARY

Porous silicon (pSi) particles show great promise for use in drug delivery and imaging applications.¹ In this work, we loaded Doxorubicin within the particles and then analyzed in vitro release kinetics. Our results will take us a step closer to understanding and engineering the controlled release of drugs from silicon microparticle scaffolds.

INTRODUCTION

Previously, it has been shown that pSi microparticles can be loaded with a variety of secondary nanoparticles including iron oxide, gadolinium, and liposomes containing siRNA.¹,² Due to the biodegradability and porous nature they are ideal as drug delivery agents especially when the drug needs to be released at a certain rate.

We are using Doxorubicin (Dox), a drug used in cancer chemotherapy, as a model drug to study loading and release with our pSi microparticle platform. Dox is an anthracycline antibiotic closely related to the natural product daunomycin. Like all anthracyclines, Dox works by intercalating DNA, with potential adverse side effects including life-threatening heart damage. It is commonly used in the treatment of a wide range of cancers, including hematological malignancies, many types of carcinoma, and soft tissue sarcomas. To study the toxicological effects of pSi-loaded Dox, we are using Draq7 as a marker of cell death. Draq7 is a new far-red fluorescent dye that only stains the nuclei in dead and permeabilized cells and is the ideal indicator as it does not stain intact, live cells.

EXPERIMENTAL METHODS

Oxidized discoidal pSi microparticles (1000 nm x 400 nm) were loaded with Dox by capillary action. Dox (2.5µM) was added to dried particles for 30 minutes, which were then washed twice to remove free drug, and re-suspended in media. Drug delivery in vitro was tested using HMVEC (human dermal microvascular endothelial cells). Cells were seeded in a 6 well plate and experiments were conducted once confluency reached ~50,000 cells per well. Draq7 (1.8nM) was added to each well for a 5 min pre-incubation period. Loaded microparticles were then added to the cells at varying cell to particle ratios including 1:10, 1:20, and 1:40.

An Olympus IX81 inverted microscope was used for 24 hr time lapse microscopy. Samples were imaged using three channels: brightfield to detect live cells, Texas Red to detect fluorescence from Doxorubicin, and Cy5 to detect Draq7. Exposure conditions for each channel were set prior to treating the cells with the loaded pSi. Cells were treated with particles once all settings were configured and the instrument was ready for image acquisition.

RESULTS AND DISCUSSION

A sample of the acquired image series is shown in Figure 1. The occurrence of apoptosis results in the fluorescence of Draq7 within the cells, providing an easy to identify marker for cell death. Figure 2 provides an overview of the timelapse with brightfield, Draq7 and Dox channels labeled as (A), (B) and (C), respectively. The timeline in column (A) shows the cells balling-up over time, a sign of cellular stress. Compromised cellular morphology and increased DRAQ7 fluorescence observed in column (B), correlated with increasing levels of Dox in column (C). For interpretation, these images are colored using a familiar “heat-map” style colour-map such that the highest pixel values are displayed in red and the lowest in blue.

Figure 1: Sample Dox image at 4 hours with three DRAQ7 signals appearing within the highlighted regions.
To verify cell death occurred as a result of internalized Dox release, each DRAQ7 signal is checked for proximity to a Dox signal in the respective frame.

Figure 2: An overview of the timelapse in each of the three channels: brightfield (A), DRAQ7 (B) and DOX (C), over intervals of 6.75 hours. Images are colored in order to accentuate the contrast for visibility and highlight features of interest. Cells shown in the brightfield image change morphology over time in response to increasing Dox levels. Accordingly, the DRAQ7 apoptotic marker becomes increasingly prevalent, indicating cell death.

As expected, higher cell death was observed for cells treated with the highest concentration of particles (1:40). Data for each well is shown in Figure 3.

Figure 3: Overview of the apoptotic events across all six well plates. Wells are either no-treatment controls (A1 and A2) or cells treated with varying ratios of Dox-loaded pSi particles - A3 (1:40), B1 (1:20) and B2 (1:10). B3 is a Dox-only control, indicating no fluorescence.

Figure 4 demonstrates normalized apoptotic events over time. With a treatment of 40 particles per cell (green curve), we observe two distinct gradients, suggesting a susceptibility to Dox-induced apoptosis at particular points in the HMVEC cell cycle.

Figure 4: A comparison of Draq7 signals for each of the three particle ratios. Each curve is normalized to its respective maximum value to allow visual comparison of their shape.

Figure 5: pSi-Dox particles were incubated with HMVEC cells for two hours, then imaged by confocal microscopy (green – phalloidin; red – Dox; blue – nuclei).

CONCLUSION
The dose of Dox used to treat HMVEC positively correlated with the rate of cell death. Delayed Draq7 signal from cells treated with encapsulated Dox was slower than free Dox due to slow release of the Dox from the endosome once internalized by the cell.

REFERENCES

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