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Communication

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Hydrogels as Intracellular Depots for Drug Delivery

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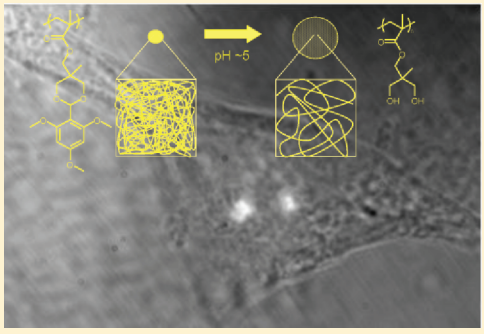
Supporting Information



ABSTRACT: The intracellular activity and drug depot characteristics of micrometer-sized hydrogels are described. The hydrogel structure is formed after cellular uptake of a solid polymeric nanoparticle that swells in response to mildly acidic conditions as it transforms from a hydrophobic to a hydrophilic structure. These nanoparticles are rapidly taken up into A549 human non-small cell lung cancer cells with 88.3 ± 0.8% of cells experiencing uptake in 24 h, undergo expansion to release encapsulated drug and can effectively deliver chemotherapeutics in vitro. The anticancer drug paclitaxel was also shown to have a 3- to 4-fold increased affinity for the expanded nanoparticle state, allowing the expansile nanoparticles to act as intracellular drug depots and concentrate the drug locally.

KEYWORDS: *hydrogel, drug delivery, nanoparticle, intracellular, depot, paclitaxel, cancer*

Hydrogels, cross-linked polymer networks that swell in aqueous solution, are widely used and investigated for both industrial and medical applications. In the field of medicine, hydrogels are used as ocular devices, drug carriers, tissue scaffolds, and sealants.[1−5] The form factor of the hydrogel can vary greatly from macroscopic contact lenses for correcting vision to nanometer-sized particles for drug delivery. Within the area of particle-based drug delivery, synthetic[6−15] and chemically modified natural[16−21] polymers are actively studied in order to address limitations of poor drug solubility, rapid in vivo clearance, low target binding, and unwanted side effects.[22−28] However, the use of a hydrogel-based particle affords an additional and unique delivery opportunity where the particle acts as an intracellular depot. As a depot, the hydrogel-based particle concentrates drug locally by partitioning the drug into the particle. Herein we investigate this concept and report the in vitro activity of hydrogel particles to deliver as well as to concentrate the anticancer drug paclitaxel within human A549 lung cancer cells.

The hydrogel structure within the cell is formed after uptake of a pH-responsive nanoparticle that transforms from a hydrophobic polymeric nanoparticle of ≈100 nm in diameter to one of ≈1000 nm in diameter once the protecting group is removed and hydroxyl functionalities are exposed, as shown in Figure 1.[28] These particles are termed expansile nanoparticles (eNPs). The eNPs are prepared following our published report using a miniemulsion polymerization method and 5-methyl-2-(2,4,6-trimethoxyphenyl)-[1,3]-5-dioxanylmethyl methacrylate, a monomer which possesses a 2,4,6-trimethoxybenzylidene acetal protecting group that is stable at neutral pH but cleaves at mildly acidic pH (see Supporting Information for details).[29,30] Scanning electron micrographs of the condensed and swollen particle states are shown in Figure 1.

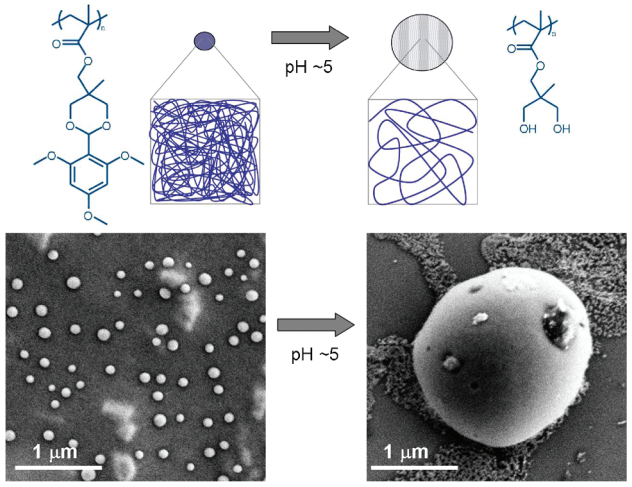


Figure 1. (top) The chemical structure of the polymeric nanoparticles and reaction leading to a swollen, expanded hydrogel state (not to scale). (bottom) Scanning electron micrographs of particles before (left) and after (right) exposure to acidic buffer conditions.

We began by confirming uptake of nanoparticles covalently modified with rhodamine (see Supporting Information) in A549 human non-small cell lung cancer cells using fluorescence microscopy to visualize the labeled eNPs. After 24 h large

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fluorescent polymeric structures of about 1000 nm were observed within the cells, consistent with a swollen state (Figure 2). Next, we quantified the eNPs cellular uptake via

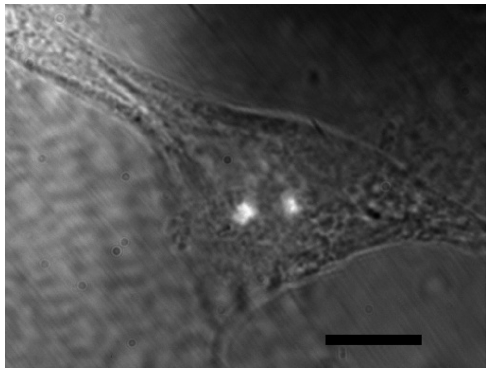

Figure 2. Gray-scale fluorescence microscopy and light contrast image of rhodamine-labeled eNPs (bright spots) in A549 cells 24 h after treatment at 37 °C. Scale bar =10 μm.

flow cytometry using the rhodamine-labeled nanoparticles. The analysis method of uptake-positive cells is described in the Supporting Information. The cellular level of nanoparticles progressively increased with incubation time at 37 °C with 88.3 ± 0.8% of cells experiencing particle uptake by 24 h (Figure 3).

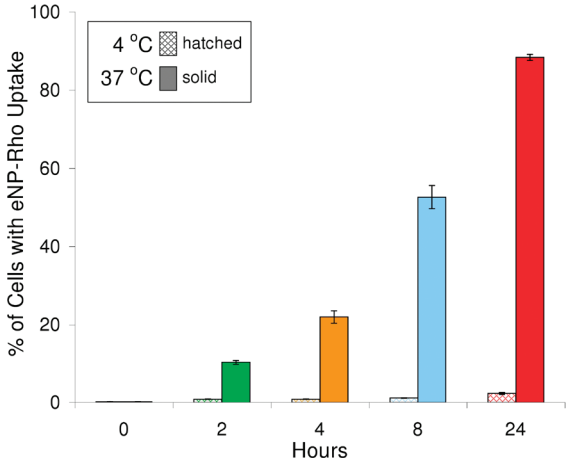

Figure 3. Uptake of rhodamine-labeled eNPs in A549 lung cancer cells as a function of time and temperature. Data displayed as mean ± SD; n = 3 (P < 0.001 at 2, 4, 8, and 24 h).

The initial rate of eNP cellular uptake (0 to 8 h) can be modeled linearly giving a rate of 6.3% of cells with eNP uptake per hour. Alternatively, when cells were incubated at 4 °C to decrease metabolic activity, cellular uptake was significantly prevented (Figure 3, P < 0.001 for all treatment times). This reduction of intracellular eNP levels at the low temperature indicates that the cellular uptake of our expansile nanoparticles is an energy-dependent process, and future studies are planned to evaluate the mechanism of NP uptake. Based on previous reports, nanoparticle charge, shape, composition, and surface chemistry are key parameters that determine cell uptake of nanoparticles via endocytosis.[31−33] For example, spherical NPs composed of hydrophobically modified chitosan are taken up by multiple endocytotic pathways, including clathrin-mediated endocytosis, caveolae-mediated endocytosis, and macropinocytosis.[34,35] Similarly, several formulations of fluorescently labeled polymer nanoparticles, including polystyrene NPs, have also been shown to utilize macropinocytosis as a primary endocytotic mechanism.[36,37]

To evaluate in vitro activity, we performed cell cytotoxicity experiments with paclitaxel-loaded eNPs (Pax-eNPs), unloaded eNPs, and paclitaxel alone against the human A549 lung cancer cell line. Upon loading the nanoparticles with paclitaxel (1 μg of polymer contains 10 ng of paclitaxel) we observed a dose dependent decrease in cell viability following 72 h of exposure with an IC_{50} value between 1 and 10 ng/mL (Figure 4) (similar

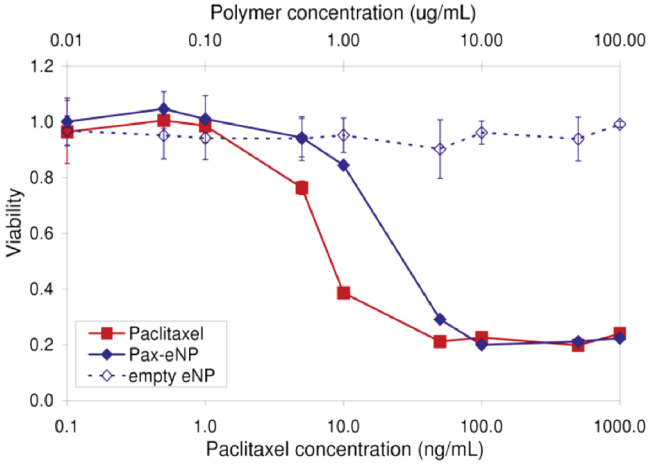

Figure 4. Percent relative viability of A549 cells following 72 h of exposure to paclitaxel, empty expansile nanoparticles (eNPs), and paclitaxel loaded expansile nanoparticles (Pax-eNPs). Data displayed as mean ± SD; n = 3.

results from a 7 day proliferation assay with SK-BR-3 breast cancer cells are shown in the Supporting Information). Coculture of A549 cells with the unloaded eNPs did not result in cytotoxicity, demonstrating that cell death was due to paclitaxel release and not exposure to the polymer itself. The shift of the curve, and corresponding increase in IC_{50} value, for the Pax-eNPs indicates that more paclitaxel must be given to afford the same cytotoxic effect. A possible explanation is that once the eNP is in the cell, it swells and becomes a hydrogel depot for paclitaxel. These nanoscale intracellular hydrogels imitate macroscopic hydrogels that have been widely used for drug delivery.[2,3,38,39] Such intracellular drug depots, we hypothesized, would decrease the immediate bioavailability of Pax leading to a higher IC_{50} initially, but would also lead to more paclitaxel residing in the cell for later use.

To quantify the ability of the eNPs to act as a paclitaxel hydrogel depot, expansile nanoparticles without drug were added to a pH 5 acetate buffer and incubated at 37 °C for 24 h to initiate swelling and transition to the hydrogel state. An aliquot of the expanded eNPs was then placed in 10,000 MWCO dialysis tubing and dialyzed against a 10 mM pH 7.4 phosphate buffer sink containing 2 μg/mL paclitaxel. After dialysis at 37 °C for 24 h, aliquots were taken of both the sink and the expanded nanoparticle phase and analyzed for paclitaxel concentration via HPLC. Figure 5 shows that, at 24 h, the paclitaxel preferentially partitioned to the swollen nanoparticle phase with a 4-fold increase of Pax in the expanded nanoparticle phase and a log D value of 0.61 ± 0.001.

In order to monitor the intracellular depot capability of the expansile nanoparticles, we performed a similar experiment to

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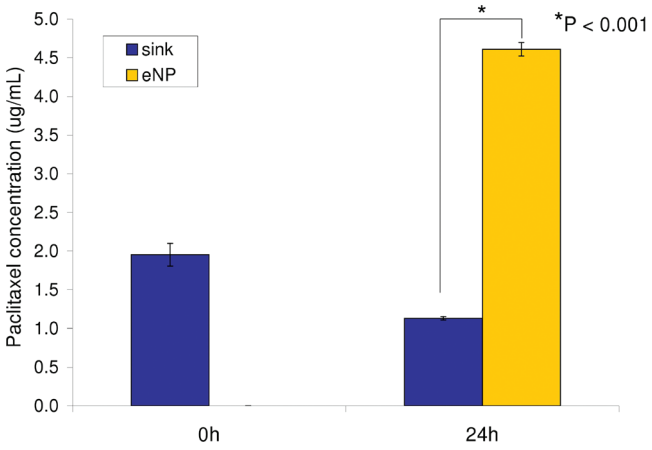

Figure 5. Paclitaxel concentration in swollen expanded nanoparticle (eNP) phase compared to aqueous buffer (sink) phase after 24 h. Data displayed as mean ± SD; n = 3.

the one above but measured uptake of paclitaxel–Oregon Green 488 conjugate (paxOG) in the A549 lung cancer cells. After plating at 24,000 cells/mL and allowing the cells to adhere for 24 h, the cells were treated for an additional 24 h with media alone, media containing eNPs, or media containing PLGA NPs (see Supporting Information). The cells were then washed with PBS, and incubated for 4 h with either media alone or media containing paclitaxel–Oregon Green. After 4 h, the media was aspirated and the cells were washed 2× with PBS before being trypsinized and replated in a new 24-well plate (see Supporting Information for details). A fluorescence plate reader was then used to measure fluorescence (λ_{ex} = 488 nm; λ_{em} = 518 nm). As shown in Figure 6, a 3-fold increase in

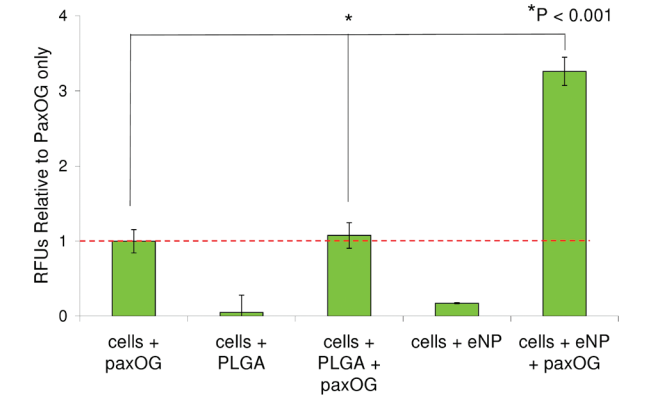

Figure 6. Relative uptake of paclitaxel–Oregon Green 488 conjugate (paxOG) in untreated cells, cells treated with conventional PLGA NPs, or cells treated with expansile nanoparticles (eNP). Data displayed as mean ± SD; n = 3.

cellular uptake of paxOG was noted in cells that were first treated with eNPs compared to both non-NP-treated cells and cells treated with conventional PLGA NPs. Finally, to visualize the nanoparticles acting as intracellular depots, we repeated the previous experiment treating A549 cells first with rhodamine-tagged unloaded particles for 24 h before treating with paxOG for an additional 4 h. Confocal microscopy images showed colocalization of the paxOG and eNPs (see representative image in Figure 7).

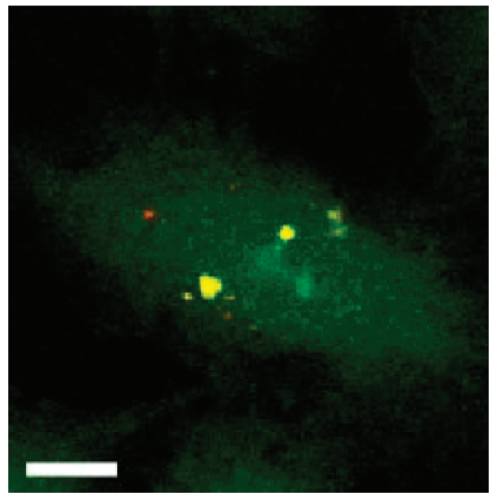

Figure 7. Confocal image showing colocalization (yellow) of rhodamine-tagged expansile nanoparticles (red) and paclitaxel–Oregon Green 488 conjugate (green) within A549 cells. Scale bar = 10 μm.

In summary, paclitaxel-loaded nanoparticles are rapidly taken up by A549 lung cancer cells and are cytotoxic in vitro against this human cancer cell line. We also show that paclitaxel possesses an affinity for the expanded nanoparticle state in aqueous buffer and that the eNPs can act as an intracellular drug depot concentrating drug locally. As the depot effect is observed for the eNPs and paclitaxel but not for PLGA NPs and paclitaxel, it suggests that other drug delivery systems might be designed, for a specific application, to match the affinity of a particular carrier to a particular drug in order to alter intracellular pharmacokinetics to improve efficacy. The in vivo implications of these results are still under investigation; however, our results with a murine lung cancer model showing that a single treatment of paclitaxel-loaded eNPs prevents establishment and delays recurrence of murine lung cancer in vivo with superiority over the conventional method of paclitaxel delivery suggest that this depot characteristic may be a critical feature.[29,40] In further support of the paclitaxel-loaded eNPs, we have recently reported their use to prevent the in vivo establishment of malignant mesothelioma.[41,42] Continued research with hydrogel-based drug delivery systems at the macro- or nanoscale will increase our understanding of how these materials function in vitro and in vivo as well as facilitate the development of new materials that meet the varied and demanding needs in chemotherapy.

■ ASSOCIATED CONTENT

Supporting Information

Details on nanoparticle synthesis, characterization and uptake. This material is available free of charge via the Internet at http://pubs.acs.org.

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