ABSTRACT SUMMARY
A multiscale chemotherapeutic drug delivery device was developed in which drug loaded micelles were encapsulated in a poly(ethylene glycol) diacrylate (PEGDA) hydrogels. The hydrogel releases drug-loaded micelles and free drug thereby extending drug release while micelles decrease drug clearance and promote drug accumulation.

INTRODUCTION
Glioblastoma multiforme is the most common and aggressive form of primary brain tumor and accounts for approximately 60% of all diagnosed brain tumors in the United States each year. Currently available treatments only minimally prolong patient mean survival time; unfortunately, these treatments also result in severe side effects.1

In an effort to better treat glioblastoma multiforme, a drug delivery device composed of hydrophobic drug (i.e. doxorubicin) loaded micelles encapsulated within a poly(ethylene glycol) diacrylate hydrogel was designed. Specially, Pluronic F-127 was chosen as the micelle-forming polymer due to its non-ionic and nontoxic properties and the ability to form stable micelles at low concentrations.2-3 Generally, Pluronics form micellar structures (of approximately 10-50 nm in size) in which lipophilic drugs may be physically incorporated.2

Poly(ethylene glycol) diacrylate (PEGDA), considered a biocompatible polymer and which has been FDA approved for use in biomedical applications, can be crosslinked to form hydrogels that slowly release trapped substances.4 This provides the added advantages of localizing the micelles near the tumor site and controlling as well as extending rate of drug release. Different molecular weight PEGDAs can form hydrogels of different mesh size, further increasing or decreasing rate of drug release. However, hydrogels are hydrophilic systems and cannot be used alone for the delivery of hydrophobic drugs. Hence, by combining micelles and hydrogels, we can alter the characteristics available in order to take advantage of the strengths of both systems as well as eliminate the weaknesses of each alone. In this way, the chemotherapy drug can be delivered to the glioblastoma and contact with healthy surrounding tissue reduced.

EXPERIMENTAL METHODS
The critical micelle concentrations of Pluronic F-127, was determined using pyrene. Micelles containing doxorubicin (DOX), indomethacin (IND) or pyrene were prepared using the oil-in-water emulsion method. An example preparation was as follows: 6 mg of doxorubicin HCl was combined with 0.5 mL of chloroform containing 20 µL of triethylamine to form doxorubicin free base. The drug solution was then added dropwise to a beaker containing 60 mg Pluronic F-127 in 120 mL of ddH2O. The entire solution was left to stir overnight in order to evaporate the organic solvent and allow for micelle formation. Micelle diameter was measured by quasi-elastic laser light scattering using a Spectramax 380 Zeta Potential/Particle Sizer. Drug loading capacity was found at differing micelle to polymer ratios (by weight). In order to encapsulate drug-loaded micelles in hydrogels, 15 mg of lyophilized, purified micelles was combined with 150 mg PEGDA (MW: 3,400, 10,000, or 20,000 g/mol) in 920 µL ddH2O. Hydrogels were polymerized by adding 35 µL 20% ammonium persulfate and 45 µL of 20% N,N,N'-N'-tetramethylenediamine. The entire solution was then poured into a mold and allowed to polymerize for 30 min at 37ºC. Hydrogel swelling ratios were determined by incubating hydrogels (of different molecular weight PEGDA and with or without micelles) in DPBS for various times. Hydrogel mesh size was measured (before or after micelle removal from hydrogel) by weighing relaxed and fully swollen hydrogels in butanol and lyophilized hydrogels in air (from Archimedes’ Principles) and then using a system of equations based on the Flory-Rehner swelling theory.4 Micelle and drug release from hydrogels was examined by incubating micelle loaded hydrogels in DPBS for 8, 24, 48, 72, 96, and 120 hours and measuring buffer fluorescence at 470/570 nm excitation/emission with a Spectramax Gemini XS spectrophotometer.

RESULTS AND DISCUSSION
Critical micelle concentration of Pluronic F-127 was determined to be 2.48 x 10⁻⁵ M which agrees with literature observations.2 Micelles when loaded with DOX or IND was found to be 50±5 nm or 20±5 nm in diameter, respectively. Empty Pluronic F-127 micelles were 10±3 nm in diameter. Drug encapsulation efficiency was highest (31% and 47% for DOX and IND, respectively) at a drug/polymer ratio of 1:10 by weight (Table 1). Therefore, for subsequent experiments, all drug-loaded micelles were made using this ratio. The highest hydrogel swelling was seen in the 20,000 Da PEGDA hydrogels followed by 10,000 Da and then 3,400 Da PEGDA (Fig. 1). Swelling ratios measured were unchanged following addition of micelles to PEGDA hydrogels. Interestingly, the measured mesh size of the hydrogels was not different if the drug-loaded micelles were removed from hydrogels or not (Fig. 2).
We also chose to load pyrene into Pluronic F-127 micelles as proof that drug-loaded micelles and not simply naked drug molecules are being released from hydrogels. Pyrene selectively fluoresces when dissolved in a non-aqueous environment, thus indicating the presence of pyrene within micelles. Pyrene release from PEGDA hydrogels encapsulating either pyrene or pyrene loaded micelles showed significant fluorescence only in the pyrene-loaded micelle groups (Fig. 3). Increasing PEGDA molecular weight resulted in increased fluorescence, and therefore, higher rates of micelles release. Similar results were observed for drug (IND or DOX) loaded micelles (Fig. 4). Micelle release from hydrogels showed a zero-order release at early times, within the first 24 hours, leveling off afterwards with continued release at a lower rate. Rate of release from hydrogel was the same for micelles containing DOX or IND.

Table 1: Drug encapsulation efficiency of DOX in Pluronic F-127 micelles with differing drug to polymer ratios. (n = 3; mean±stdev)

<table>
<thead>
<tr>
<th>Drug to Polymer (by weight)</th>
<th>Dox Encapsulation Efficiency</th>
<th>IND Encapsulation Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 10</td>
<td>30.8±7.4</td>
<td>47.4% ± 6.96</td>
</tr>
<tr>
<td>1 to 4</td>
<td>16.2±3.4</td>
<td>29.3% ± 5.99</td>
</tr>
<tr>
<td>1 to 2</td>
<td>19.7±3.5</td>
<td>21.6%± 2.38</td>
</tr>
<tr>
<td>3 to 4</td>
<td>21.1±1.7</td>
<td>15.4%± 3.44</td>
</tr>
<tr>
<td>1 to 1</td>
<td>25.6±1.1</td>
<td>14.6%± 4.67</td>
</tr>
</tbody>
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Figure 1: Dynamic swelling ratios of PEGDA hydrogels in DPBS. Swelling ratio is the weight of swollen hydrogel minus the weight of hydrogel at a given time divided by the weight of hydrogel at time zero.

Figure 2: Mesh size of hydrogels. The mesh size was calculated with DOX-loaded (red) or Indo-loaded (blue) micelles present (crossed bars) or after micelle removal (solid bars). Each bar represents the average plus or minus (±) the standard deviation of three independent experiments.

Figure 3: Release of pyrene or pyrene-loaded Pluronic F-127 micelles from 3,400, 10,000, or 20,000 Da molecular weight PEGDA hydrogels.

Figure 4: DOX-loaded (left) or IND-loaded (right) Pluronic F-127 micelle release from 3,400 , 10,000 , and 20,000 Da molecular weight PEGDA hydrogels.

CONCLUSIONS

A drug delivery system consisting of DOX-Pluronic F-127 micelles encapsulated within a PEGDA hydrogel was developed for the treatment of solid tumors (with a focus on glioblastoma multiforme). We present both micelle and hydrogel characteristics in this system and demonstrate the ability of PEGDA hydrogels to release drug-loaded Pluronic F-127 micelles over an extended period of time.

REFERENCES


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