ABSTRACT SUMMARY
A multiscale chemotherapeutic drug delivery device was developed in which drug loaded micelles were encapsulated in a polyethylene glycol diacrylate (PEGDA) hydrogels. The hydrogel releases the micelles which extend 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 in human and accounts for approximately 60% of all diagnosed brain tumors in the United States each year. Current available treatments such as radiation, resection, and repeated intravenous injections of chemotherapeutic agents are only able to minimally prolong patient mean survival time; unfortunately, these treatments often result in severe side effects. Likewise, it is also extremely difficult to deliver hydrophobic drugs through the blood-brain barrier.

In an effort to better treat glioblastoma multiforme, a drug delivery device composed of hydrophobic chemotherapy drug (i.e. doxorubicin) loaded Pluronic F-127 micelles encapsulated within a polyethylene diacrylate scaffold (hydrogel) was designed. Pluronic F-127 is a nontoxic, nonionic surfactant polyol that can form micellar structures (of approximately 50 nm in size) in which lipophilic drugs may be physically incorporated. 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. By combining the advantages of a micelle and hydrogel system, the hydrogel containing micelles can be inserted via a catheter at/near the brain tumor site and act to both localize the micelles and be able to slowly release lipophilic drugs over an extended period of time. In this way, the chemotherapy drug can be delivered to the glioblastoma and contact with healthy surrounding tissue reduced.

EXPERIMENTAL METHODS
The critical micelle concentration of Pluronic F-127 was determined using pyrene. Micelles containing either doxorubicin 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 drop wise to a beaker containing 60 mg Pluronic F-127 in 120 mL of deionized water under rapid stirring. The entire solution was left to stir overnight in order to evaporate all of the organic solvent and allow for micelle formation. Micelle diameter was measured by quasi-elastic laser light scattering using a Niconmp 380 Zeta Potential/Particle Sizer in ddH2O and drug loading capacity was also determined 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: 20,000 Da) in 920 µL ddH2O. Hydrogels were polymerized by adding 35 µL 20% ammonium persulfate and 45 µL of 20% N-N'-N'-N'tetramethylenediamine to the PEGDA, micelle mixture. The entire solution was then poured into a mold and allowed to polymerize for 30 min at 37ºC. Hydrogel swelling ratios (3 experimental groups: PEGDA hydrogels and PEGDA hydrogels encapsulating Pluronic micelles, and PEGDA hydrogels encapsulating DOX loaded micelles) were determined by incubating relaxed hydrogels in DPBS for various times. Micelle and drug release from hydrogels were 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 GeminiXS fluorescence plate spectrophotometer. Human glioblastoma cell line U251-MG was used for in-vitro studies: ~22,000 cells/cm² for 24 hour incubation time before addition of treatment. The MTS assay was then conducted to test for cell viability.

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. Micelles when loaded with doxorubicin were found to be ~50 nm in diameter compared with empty Pluronic F-127 micelles which were 10-20 nm in diameter. Micelle drug encapsulation efficiency was highest (~31%) at a drug/polymer ratio of 1:10 by weight (table 1). Therefore, for subsequent experiments, all DOX-loaded micelles were made using this ratio. Swelling ratios (q) did not change despite adding micelles to PEGDA hydrogels (fig. 1). Doxorubicin micelle release from PEGDA hydrogels was rapid within the first 8 hours of incubation with slower steady release over the next 120 hours (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. Release from PEGDA hydrogels encapsulating either pyrene or pyrene loaded micelles...
show significant fluorescence only in the pyrene loaded micelle group (fig. 3).

Glioma cells (fig. 4) showed significant growth inhibition in groups treated with either DOX micelle or DOX HCl loaded hydrogels or either free DOX micelle or DOX HCl. DOX loaded micelles are just as cytotoxic to cancer cells grown in a 2-D environment as free DOX HCl. Future studies will focus on testing this system on tumoroids (growing U251-MG cells in a 3-D environment such as a collagen scaffold) and in-vivo.

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<thead>
<tr>
<th>Drug:Polymer Ratio (by mg weight)</th>
<th>Drug Encapsulation Efficiency</th>
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<tbody>
<tr>
<td>1:10</td>
<td>30.8±7.4</td>
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<tr>
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<td>25.6±1.1</td>
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Table 1: Drug encapsulation efficiency of DOX in Pluronic F-127 micelles with differing drug/polymer ratios.

**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). In-vitro studies have shown micelle release from hydrogels and significant cellular cytotoxicity upon treatment.

**REFERENCES:**

**ACKNOWLEDGEMENTS:**
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