6-Methoxyethylamino Numonafide (MEAN) Eluting Microspheres for Catheter-Directed Delivery to Liver Tumors

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ABSTRACT SUMMARY
We synthesized 6-methoxyethylamino numonafide (MEAN)-magnetic microspheres to be used for transcatheter-targeted HCC chemotherapy using a microfluidic technique. Microfluidic methods effectively produced hydrophilic, deformable, and non-aggregated biodegradable MEAN magnetic microspheres. These microspheres offer the benefits of precise controlled release of MEAN chemotherapeutic agent into the HCC tumor bed by transcatheter targeting and also visibility with MR imaging.

EXPERIMENTAL METHODS
MEAN drug was synthesized as reported in previous publications.¹,² USPIO clusters were synthesized using a high temperature hydrolysis reaction. Microfluidic chips with a channel dimension of 50–120 um in each depth and width were fabricated by conventional soft lithography techniques. A droplet gelation process was adopted to produce MEAN loaded magnetic alginate microspheres using the microfluidic chip. The oil used as the continuous phase was composed of n’-hexadecane and span 80 (2% w/w). Aqueous solutions of sodium alginate (2% w/w), USPIO clusters and MEAN were prepared for the dispersed phase. The generated droplets were collected in a calcium chloride reservoir for external gelation experiments. Finally, the microspheres were freeze-dried in lyophillizer (Labconco Freezone 6, MO, USA) after washing. Drug elution studies for each set of microspheres were performed to compare MEAN release kinetics of MEAN-magnetic microspheres incorporating various amounts of USPIO clusters (0~5 wt%) at 37 °C. MR relaxivities of the samples were investigated using a 7 Tesla MRI scanner (BioSpec, Bruker, Billerica, MA, USA). The HCC cell growth inhibition effects of MEAN-magnetic microspheres were investigated by treating McArH7777 HCC cell line with free MEAN and MEAN-magnetic microspheres at various concentrations (1.25–50 uM of MEAN) and treatment times of 8, 24 and 40 hours using MTT assay. Apoptosis and necrosis were determined by propidium iodide and Annexin-V. For in vivo study, male Sprague Dawley rats were implanted with 5x10⁶ McArH7777 cells.
and a tumor was allowed to grow for 7 days, in which the rats then underwent a procedure to have the proper hepatic artery catheterized via the gastroduodenal artery and infused with the microspheres. In addition, pre and post-procedural MRI images were acquired with T2 and T2* sequences to allow for visualization of the intra-hepatic biodistribution of the MEAN-magnetic microspheres after the infusion. After 72 hours, the rats were sacrificed and the liver tissue was harvested and sectioned for Prussian blue staining for iron as a secondary confirmation of microsphere delivery.

RESULTS AND DISCUSSION

Microfluidic methods effectively produced hydrophilic, deformable, and non-aggregated biodegradable alginate microspheres with a mono size range of 30~70 µm (size dependent upon channel size and flow rates). Encapsulation of MEAN and USPIO clusters in the alginate microspheres was confirmed with fluorescent microscopy. The USPIO clusters embedded into the alginate matrix controlled drug release rates and prevented initial burst drug release. Strong T2-weighted image contrast was achieved due to the USPIO clusters within these microsphere drug carriers.

In vivo results indicated that microspheres could be delivered locally to liver tumors intra-arterially with a good success rate in an ideal rat HCC model. The microsphere delivery could be monitored as the microspheres were depicted as punctate regions of darkening near or around the tumor, which could be additionally confirmed with Prussian blue staining for iron.

CONCLUSION

The potential benefits of these MEAN-magnetic microspheres should be considerable. These microfluidic fabricated magnetic microspheres can provide a precise controlled release of MEAN chemotherapeutic agent into the HCC tumor bed following transcatheter delivery; these microspheres also offer the potential for non-invasive visualization with MRI for confirmation of delivery.

REFERENCES


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