MRI Contrast Behavior of Ferritin-associated Gadolinium Liposomes on Glioma Tumors

Kari Duck1, AB Madhankumar2, Qing Yang3, Becky Slagle-Webb4, Nicholas Drayer5 and James Connor2

1. Biochemistry and Molecular Biology Program, Ursinus College, Collegeville, Pennsylvania
2. Department of Neurosurgery, Milton S. Hershey Medical Center, Penn State University, Hershey, Pennsylvania
3. Department of Radiology, Milton S. Hershey Medical Center, Penn State University, Hershey, Pennsylvania
4. Department of Biology, Lock Haven University, Lock Haven, Pennsylvania

Introduction
Nuclear magnetic resonance imaging has played a crucial role in developing a better understanding of tumor formation. In this research, we attempted to improve the quality and capacity of imaging brain tumors using a previously identified nanovehicle transport system (NVT). Gadolinium (Gd), a common MRI contrast agent used in visualizing brain tumors, was encapsulated in a liposome and then used to treat U251 glioma cells in vitro and in vivo models as well as in an in vivo model. In addition to a standard monolayer cell model, we implemented a spheroid model in which we would be able to see how the liposomes penetrated into the spheroid core. We further hypothesized that use of H-Ferritin (HFn) in the liposome would create better contrast. Thus, we generated liposomes in which both Gd and HFn were encapsulated into a liposome. Results indicated that the liposomes containing Gd and HFn were suitable for the in vivo model based on their non-toxic nature. When optimized, this Gd transport system will enable radiologists and surgeons to better visualize the entire tumor so that the best decisions can be made with regards to life-saving measures.

Materials and Methods
Liposomes were made by reconstituting a lipid film in a 10% solution of Gd and RBS or Gd, HFn and RBS based on the type of liposome being made. Cytotoxicity data was obtained using a MTS/PMS assay. For the MRI study, cells were treated with the liposome, suspended in 1% sepiolite agarose and imaged using T1 relaxation. Nude mice were injected with U251 glioma cells and tumors were allowed to develop. The mice were then treated with either the Gd liposome or the Gd + HFn liposome and imaged again to determine if there was improved contrast in the latter condition.

Results
Cytotoxicity Data for Gd-containing Liposomes

Picture 1: Cytotoxicity data for three liposome formulations. Data indicates that the liposomes containing gadolinium only as well as liposomes containing both gadolinium and polylysine were extremely toxic to U251 cells.

Table 1. Charge and particle size analysis. The liposomes containing only Gd are the most negative and have the smallest particle size. Liposomes containing both Gd and polylysine have a slightly larger charge and size than those containing only Gd. All samples were kept at 4°C.

<table>
<thead>
<tr>
<th>Liposome Type</th>
<th>Charge (zeta potential)</th>
<th>Particle Size (nm)</th>
<th>Polydispersity Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gd</td>
<td>-19.7 ± 2.81</td>
<td>100</td>
<td>0.065</td>
</tr>
<tr>
<td>Gd + HFn</td>
<td>-4.95 ± 1.02</td>
<td>123</td>
<td>0.140</td>
</tr>
<tr>
<td>Gd + Polylysine</td>
<td>11.8 ± 1.51</td>
<td>132</td>
<td>0.122</td>
</tr>
</tbody>
</table>

Figure 3. MRI analysis of Gd and Gd + HFn liposomes. Timecourse data showing very little change in relaxation.

Discussion and Conclusions

It is suggested that liposomes containing only Gd as well as those containing both Gd and HFn are not fully developed. Additional factors, such as the addition of more Gd to be encapsulated and increases in both Gd and HFn, may improve the results.

3. More concentrations need to be tested in order to determine the optimal contrast.

Table 2. Atomic absorption data. Concentrations of each liposome type were determined using atomic absorption. This data indicates that gadolinium is better incorporated into liposomes containing either H-Ferritin or polylysine.

<table>
<thead>
<tr>
<th>Liposome Type</th>
<th>Absorbance</th>
<th>Gd Concentration (ppm)</th>
<th>Actual Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gd</td>
<td>0.641</td>
<td>72.95</td>
<td>93.84</td>
</tr>
<tr>
<td>Gd + HFn</td>
<td>0.175</td>
<td>50.75</td>
<td>81.87</td>
</tr>
<tr>
<td>Gd + Polylysine</td>
<td>0.354</td>
<td>60.42</td>
<td>93.84</td>
</tr>
</tbody>
</table>

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References

Acknowledgments
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Benjamin M. S. McKee, PhD; co-Directors: Cheng Dong, PhD, Alan Snyder, PhD, and Erin Sheets, PhD; Program Director: McDonald; www.bbsi.psu.edu
Penn State Biomaterials and Bionanotechnology Summer Institute

Engineering of Cross-Linked Biomaterials for Controlled Release of Therapeutics

Bryan Streitmatter,1,2 Guant P. Mora,3 Christopher Stelhlik,4 William Watts,5,6 and Taif Lu Liew,4,7,8
Penn State Biomaterials and Bionanotechnology Summer Institute (BBSI), 1 Cornell University, 1 Department of Surgery, 2 Biomedical Engineering, 4 Materials Science and Engineering, 5 Pennsylvania State University, 500 University Drive, Hershey, Pennsylvania 17033, U.S.A.

Abstract

Minimizing thrombus formation is of primary importance in the execution of major surgeries. Two important examples are the implantation of biomedical devices and open heart surgeries. To prevent thrombus formation on the surface of the implanted Polyurethaneurethane (PUU) blood-contacting sarms of the Penn State Lionheart, we have envisioned two novel strategies. 1) Formation of a hydrogel-entrapped, pseudo-recombinant layer on the PUU surface, and 2) sustained release of heparin from nanoparticles embedded within the PUU matrix. For the first strategy involves grafting cross-linked PEG onto the PUU surface. Ultimately, the pore size and thickness of the cross-linked PEG could be tailored to foster formation of an entrapped pseudo-recombinant layer. The chemical structures of the resulting PEG grafted PUU films are characterized by a Fourier transform infrared spectroscopy technique. In the second strategy, thermoresponsive-co-biodegradable nanogels are developed for the sustained release of heparin, and subsequently embedded in the PUU blood acsy. In an attempt to prevent thrombus formation in open heart surgeries, a hydrogel implantation strategy has been envisioned. A hydrogel-entrapped heparin could be implanted into the heart tissue, where it will release the drug directly to the heart. The efficacy and duration with which the nano and hydrogels can release heparin and investigated with a toluene blue assay. Completion of this project will have significant impact not only on the development of Penn State adult and pediatric cardiac repair devices, but also on improving hemocompatibility of PUU and other blood-contacting biomaterials and thrombosis prevention in major surgeries.

Materials and Methods

Strategy One: Hydrogel Grafting

- Polyurethaneurethane (PUU) Blood-Contacting Sarms
- Cross-linked PEG Grafting
- Hydrogel-Entrapped Pseudo-Recombinant Layer

Strategy Two: Nanogel Embedding

- PEGMA + degree of polymerization (DP) 5 / degree of substitution (DS) 17
- Dextran/PDLLA macromer + IRGACURE photoinitiator solution UV exposed at 365nm/2000mW/cm2 exposed for 1hr under vigorous stirring to allow cross-linking
- PBS (pH 7.4) immersion at 37°C to allow low molecular weight heparin release; release quantified by Toluene Blue UV Absorbance Assay

Strategy Three: Hydrogel Implantation

- PEGMA + DP 3/ DS 5.5, DP 3/ DS 12.5; or DP 6/ DS 17
- Dextran/PDLLA macromer + IRGACURE photoinitiator solution UV exposed at 365nm/2000mW/cm2 for 5 min. in cylindrical latex mold
- PBS (pH 7.4) immersion at 37°C to allow heparin release; release quantified by Toluene Blue UV Absorbance Assay

Experimental Findings

Strategy One: ATR-FTIR Characterization of PUU Films

- Thermo Model Avanta 370 FTIR

Summary

- Both catalysts yielded only intermittent success in functionalizing PUU surfaces
- Hydrogels can release regular molecular weight heparin or low molecular weight heparin for at least 16 or 10 days, respectively

Future Directions

- Investigate alternative chemistries for strategy one
- Start release profile studies for Strategy Two
- Continue release profile studies for Strategy Three
- In vivo blood-contacting experiments for all strategies (e.g., hemolysis)

References


Acknowledgments

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Culture on Specific Nanoscale Topographies Selects for Subpopulations of Stem Cells with Increased Osteogenic Potential

Joshua D. Salvi, Jung Yul Lim, Christopher Niyibizi, Yue Zhang, Henry J. Donahue

Engineering, The Pennsylvania State University, University Park. A
Skeletal Sciences, Department of Orthopaedics and Rehabilitation.
Of Medicine, Pennsylvania State University, Hershey, PA.
Drug Delivery across the BBB

Direct delivery to the brain: Invasive surgery

BBB disruption: Unexpected toxicity to the brain

Delivery across the BBB: Carriers or transporters