Polyelectrolyte Negatively Charged Hydrogel for Doxorubicin Delivery

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Statement of Purpose: Doxorubicin (DOX) is a chemotherapeutic agent used in the treatment of a wide variety of cancers. Even short-term use of DOX can induce cardiotoxicity, and long-term use can lead to congestive heart failure due to the accumulation of the drug and its derivatives. These harmful side effects pose significant challenges to the safety and efficacy of anti-cancer treatments which may be overcome through the development of a controlled drug delivery system. Oligo(poly(ethylene glycol)) fumarate (OPF) is an injectable, cross-linkable, and biocompatible macromer that has easily-modulated mechanical and degradation properties.[1] It has been shown that OPF can be copolymerized with the positively-charged monomer [2-(methacryloyloxy) ethyl]-trimethylammonium chloride (MAETAC) to produce positively-charged polyelectrolyte hydrogels.[2] It has also been shown that OPF hydrogels can be used for sustained DNA delivery to bone cells.[3] In the present study we develop a protocol for fabricating negatively-charged hydrogel discs and microspheres through incorporation of the negatively-charged monomer sodium methacrylate (SMA), and evaluate the potential applications of these hydrogels in a controlled drug delivery system for DOX.

Methods: ***OPF Hydrogel Disc Fabrication:*** OPF was synthesized from purified PEG with initial Mn of 10,000 g mol^{-1} according to a previously published method.[1] OPF macromer (final concentration 33% w/w), photoinitiator Irgacure-2959 (0.05% w/w), co-monomer N-vinyl pyrrolidinone (0.33% w/w), and SMA (0, 5, 10, 20, 30% w/w) were dissolved in deionized water. The reaction mixture was placed between glass slides with 1 mm spacer height and polymerized under UV light (λ = 365 nm) for 30 minutes.

OPF Hydrogel Microsphere Fabrication: OPF macromer (final concentration 33% w/w), co-monomer N-vinyl pyrrolidinone (0.33% w/w), and SMA (0, 5, 10, 20, 30% w/w) were dissolved in deionized water. The reaction mixture was emulsified in mineral oil and polymerized using thermal radical initiators ammonium persulfate and tetramethylethylenediamine for 30 minutes at 40°C.

DOX Absorption and Release: Discs and microspheres were incubated in an aqueous DOX solution (5 µg/mL) for 24 hours at 37°C. DOX release in PBS was measured over 7 days.

Cell Viability Study: MG63 cells were incubated for 3 days at 37°C with plain or DOX-loaded (100 µg) microspheres. An MTS assay was performed to determine cell viability.

Results: Negatively charged hydrogels were characterized after fabrication using FT-IR spectroscopy and their swelling ratio was measured by gravimetrical method in deionized water and PBS. Our data showed that negatively charged hydrogel swelling ratios increased with increasing charge density in water and were significantly greater in water than in PBS ($p<0.05$) indicating the electrolyte properties of the hydrogels. Microsphere swelling ratios in both water and PBS were significantly greater than those of the discs ($p<0.05$). DOX in aqueous solution was absorbed to the surface of hydrogels due to electrostatic interaction between positively charged DOX and negatively charged hydrogels. DOX absorption increased with increasing charge density in both hydrogel discs and microspheres. DOX release from hydrogels was measured using colorimetric method at 490 nm. Neutral discs and neutral and 5% charged microspheres exhibited a burst release profile. However, discs and microspheres of higher charge density exhibited controlled release profiles over the 7 day time period.

Figure 1 shows morphology of hydrogel microspheres after lyophilization (upper panel) using SEM. The size of microspheres was in the range of 5-50 µm. Figure 1 (lower panel) shows microspheres with different charge density after swelling and loading with DOX.

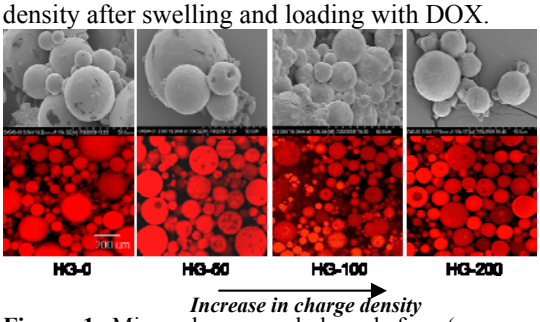

Figure 1. Microsphere morphology before (upper panel) and after (lower panel) swelling.

Figure 2 reveals the ability to induce cell death by Dox delivered from hydrogel microspheres. Induction of cell death was statistically significant in all experimental groups.

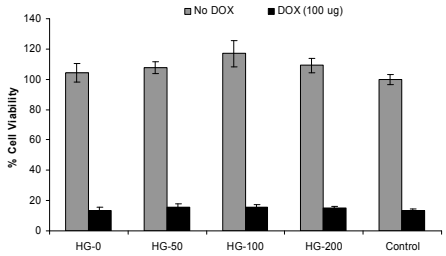

Figure 2. Anti-tumor effect of released DOX

Conclusions: Our results show that negatively-charged hydrogels exhibit unique electrolyte properties. DOX absorption and release profiles can be modulated by changing negative charge density in OPF hydrogels. DOX released from negatively-charged OPF hydrogels maintained its anti-tumor effects.

References:

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2. Dadsetan, M. et al., *Biomaterials.* ***2009***, 30, 3874.
3. Jo, S. et al., *Biomacromolecules.* **2001**, 2 , 255.

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