Evaluation of novel intratumoural oxygen delivery platforms for improving radiotherapy response in hypoxic solid tumours

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Background: Radiation has long been a powerful weapon against solid tumours. Oxygen is recognized as a potent chemical radiosensitizer that enhances the cell killing efficiency of radiation. However, as it is common for solid tumours to be hypoxic, tumours present with resistance to radiation. Known for being extremely hypoxic, pancreatic ductal adenocarcinoma (PDAC) remains one of the worst among solid tumours. Methods to increase oxygen level in PDAC are therefore desirable. Perfluorocarbons (PFCs) have emerged as a promising vehicle for dissolving significant amount of oxygen. Our project aims to develop oxygen-loaded perfluorocarbons (PFCs) nanoemulsions for direct intratumoural injection to boost patient response to radiation by alleviating hypoxia in PDAC.

Methods: PFCs-based nanoemulsions incorporating a commercial iodinated contrast agent (Visipaque™, abbr. VP) were prepared via sonication. The droplet size and polydispersity index were characterized using Malvern's Zetasizer. The viscosity was assessed using a HR-1 Discovery Hybrid Rheometer with the relationship between viscosity and oxygen storage capacity evaluated. Oxygen loading and release was evaluated by Firesting O_2 optical oxygen meter. The imaging potential and distribution of nanoemulsions were preliminarily assessed on ex vivo mouse livers via Quantum GX2 MicroCT Imaging System. The cytotoxicity of PFCs nanoemulsions to BXPC3 cells receiving radiation +/- was evaluated using MTT assay and γ-H2AX probed Western Blot.

Results: Two lead formulations (NE_15VP and NE_30VP) showed stability for up to 6 month with a droplet size range of 140-160nm and a PDI range of 0.2-0.3. The presence of increased VP resulted in increased viscosity, with NE_30VP more viscous than NE_15VP. NE_15VP and NE_30VP were oxygenated to over 600 mmHg, with oxygen in NE_30VP released slower than in NE_15VP. NE_30VP further incorporating viscosity enhancing (VE1 and VE2) agents reduce oxygen loading but also significantly slow down oxygen release. The presence of VP enabled our nanoemulsions to be successfully visualised via microCT, with a greater retention at the intratumoural injection site as viscosity increased. For in vitro study, NE_15VP and NE_30VP showed almost no toxicity to BxPC3 cells. BxPC3 cells treated with oxygenized nanoemulsions in combination with radiation exhibited more DNA damage in preliminary experiments. However, monolayer cell culture has been criticized for not being a reliable predictor of treatment response in vivo. Hence, we established three-dimensional GELMA to form BxPC3 spheroids. GELMA with stiffness of around 3.9 kPa was successfully established which provided excellent viability of 3D spheroids for greater than 69 days, offering an improved in vitro approach for predicting radiation response of our oxygen-loading PFCs nanoemulsions in the future.

Conclusions: Two stable nanoemulsions incorporating a commercial imaging agent have been successfully developed. Increasing viscosity slows down oxygen release and increases nanoemulsion's retention at site of injection, providing a new approach for oxygen delivery to hypoxia tumours. Our nanoemulsions effectively enhance radiation response to cells. GELMA will offer an improved in vitro approach for predicting radiation response of our oxygen-loading PFCs nanoemulsions in the future.