Published in final edited form as:

Proc SPIE Int Soc Opt Eng. 2013 February 26; 8584: 858411–. doi:10.1117/12.2007310.

Development of a biodegradable iron oxide nanoparticle gel for tumor bed therapy

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Abstract

Treatments of the post-operative surgical bed have proven appealing as the majority of cancer recurrence following tumor resection occurs at the tumor margin. A novel, biodegradable pullulan-based gel infused with magnetic iron oxide nanoparticles (IONP) is presented here for surgical bed administration followed by hyperthermia therapy via alternating magnetic field (AMF) activation. Pullulan is a water soluble, film-forming starch polymer that degrades at the postoperative wound site to deliver the IONP payload, targeting the remaining cancer cells. Different gel formulations containing various % wt of pullulan were tested for IONP elution. Elution levels and amount of gel degradation were measured by immersing the gel in de-ionized water for one hour then measuring particle concentrations in the supernatant and the mass of the remaining gel formulation. The most promising gel formulations will be tested in a murine model of surgical bed resection to assess *in vivo* gel dissolution, IONP cell uptake kinetics via histology and TEM analysis, and heating capability of the gel with AMF exposure.

Keywords

iron oxide nanoparticles (IONP); biodegradable; gel; cancer; hyperthermia; surgical bed

1. IDENTIFICATION OF PROBLEM

The use of iron oxide nanoparticles (IONP) for targeted hyperthermia cancer therapy via alternating magnetic field (AMF) activation has shown great promise in initial pre-clinical testing^{1,2}. IONPs have been used extensively in *in vitro* and *in vivo* experiments with mice and have been shown to be both safe and effective.

For nanoparticle hyperthermia to work, IONPs, which are coated with a biocompatible surfactant, such as Dextran or polyethylene glycol (PEG), need to get taken up by cancer cells and aggregate within the cells. Once the IONP are aggregated within the tumor cells, IONPs are exposed to an alternating magnetic field between 30 and 300 kHz. This field puts

IONPs in a hysteresis loop of alternating magnetism, which causes localized hyperthermia, thus damaging and killing cells containing the nanoparticles. While the exact mechanism for cytotoxicity is still debated with regards to whether the IONPs are damaging and killing cells through hyperthermia or through damage induced from mechanical vibrations, the results are clear: IONPs offer an effective localized approach to treatment of cancer cells, maximizing the therapeutic ratio.

Although several groups are investigating strategies to administer IONP systemically, the current method of delivery *in vivo* is direct injection of IONPs into the tumor. While this has been shown to be effective in subcutaneous mouse tumors³, it has limitations for widespread clinical applications. In particular, it is difficult to deliver IONPs, into a deep or diffuse tumor, (see Figure 1 below), to hematologic or metastatic disease, or onto a tumor bed.

IONP hyperthermia treatment can be used adjunctively to improve the outcomes of cancer patients by increasing the efficacy of conventional treatments such as radiotherapy, chemotherapy, and surgical resection. Although the goal of most surgical treatments is complete resection of the tumor, in some cases, this goal is not achievable because of the encasement of tumor around vital structures. Even in cases in which total resection is possible, residual cancer cells may be left on the margins of a tumor bed and may result in treatment failure with local recurrence. Treating the tumor bed with intraoperative radiation or chemotherapy has been used, but the therapeutic ratio is low because of the unacceptable damage to local normal tissue. IONP hyperthermia treatment to the tumor bed has the potential to treat known or suspected residual cancer cells to improve the efficacy of surgical treatment..

An improved and targeted delivery vehicle for IONPs would be an ideal solution for both treating residual cancer cells on a tumor bed and increasing the application, efficacy and therapeutic ratio of IONPs.

2. PROPOSED SOLUTION

To more directly and specifically deliver IONPs to a tumor bed, we created a biocompatible, deformable gel infused with IONPs that could be used as an effective vehicle for administration of IONPs into cancer cells in a tumor bed. This gel had to meet the following specifications:

2.1 Biocompatibility

As this gel will be applied in tumor beds, which can be located at any anatomical site, it must not be rejected by the body or produce any adverse reactions. It must be able to be sterilized as it will be placed in sterile surgical beds.

2.2 Toxicity

The components of the gel must have minimal tissue toxicity both locally and systemically. Both short-term and long-term toxicity will be considered.

2.3 Deformability

The gel must be able to integrate in to a diffuse, aberrant tumor bed. It must adhere directly to the tissue and have sufficient mechanical properties to remain in place.

2.4 Temperature Consistency

The gel must maintain mechanical properties at temperatures ranging from room temperature, to normal body temperature(37 C) to maximum hyperthermia temperatures (43-47 C).

2.5 High IONP Concentration Capacity

The gel must maximize the IONP delivery per unit volume; the goal is to suspend the same concentration of IONP in the gel such that it has the same mole fraction as it would have in H₂O or phosphate-buffered saline (PBS).

2.6 Stability

The gel must remain chemically stable both within the body and on the shelf.

2.7 IONP Release Capability

The gel must degrade effectively and rapidly such that it is able to elude IONPs into surrounding cancer tissue

A gel meeting these specifications would be a unique and effective delivery agent for IONP cancer therapy on the tumor bed.

3. GEL DEVELOPMENT

3.1 Testing Formulations

Numerous possible compounds in various combinations were tested for their efficacy as an IONP gel; the compounds used were NaHCO₃, gelatin, glycerin, NaCl, guar gum, xanthan gum, methyl cellulose, and a polysaccharide powder known as pullulan. First, these gels were examined qualitatively to see if, by inspection, they met our deformability specification. We found that thickening ½ mL of IONPs with a 2 tablespoon xanthan gum mixture offered a gel that was not viscous enough for our needs. We also found that adding ½ mL of IONPs to a mixture of ¾ cup NaHCO₃, ¼ cup NaCl, and ¼ oz. gelatin proved to have too low of a viscosity. Adding ¼ mL IONPs to ¾ cup NaHCO₃, ¼ cup NaCl, H₂O, and 12 teaspoons of glycerin produced the same undesirable result. 2 tablespoons of guar gum with ½ mL of IONPs and 2 tablespoons of xanthan gum with ½ mL of IONPs were more viscous but tended to break apart into separate chunks (see Figure 2 below). Biocompatibility of xanthan gum and guar gum was also questionable. All of these compounds, however, did have shelf stability.

3.2 Effectiveness of a Polysaccharide

We began to pursue using a polysaccharide formulation when we considered its capability as both a film-forming polymer and as a compound that can be compatible with starch-coated

IONPs. Pullulan emerged as the ideal polysaccharide for use given its film-forming capabilities and its compatibility with starch-coated IONPs. We conducted a similar series of tests as we did with the other formulations to check for deformability by mixing pullulan with PBS. We found that 1 g of pullulan and 9 mL PBS, after incubation for 24 hours at 37C, produced a gel with a viscosity that was too low; however, a 3 g pullulan and 7 mL PBS gel, after undergoing the same incubation process, had an ideal viscosity to be an effective vehicle for IONP delivery.

Next, we tested pullulan's efficacy in forming a biodegradable gel with three different types of IONPs. We tested with 20 nm nanomag-D SPIO Dextran IONPs at a concentration of 108 mg/mL, 80 nm BNF Dextran IONPs at a concentration of 38 mg/mL and 100 nm nanomag-D SPIO Dextran at a concentration of 10 mg/mL. We found that, regardless of IONP type, pullulan formed a thick, high viscosity hydrogel with the IONPs, and these gels were seen to be stable (see Figure 3 on following page).

With these specifications of deformability, compatibility with the IONPs, high IONP concentration capacity and stability met, we then tested the biodegradability and IONP release capacity of this gel. In order to best understand the biodegradability of the gel and its elution properties, we added 10 mLs of H₂O to each of the three IONP gel formulations we created. These samples were stored at 37C and incubated for 1 hour. After 1 hour incubation, the gels were first examined by inspection to determine efficacy of biodegradability. All three gel formulations showed significant degradation after 1 hour (see Figure 4 on the following page).

The IONP liquid from each gel formulation was then removed from the gel via pipette and put in separate 15 mL conical tubes for elution testing. The concentrations of IONPs that had eluded into the sample liquids were tested. Absorbance spectroscopy was used to determine the concentration of nanoparticles by comparing the absorbance value of the samples to known baseline values of solutions with different concentrations of nanoparticles. The elution data from absorbance spectroscopy is being processed.

10 mL of H₂O was then added to the gel formulations that remained after the IONP liquid was removed; the gel was incubated overnight at 37C. After overnight incubation, the entire gel formulation was fully dissolved with the entire gel film left on the bottom of the tray. This shows that the pullulan IONP gel formulation has the ability to degrade rapidly and both carry and elude high concentrations of IONPs.

Moreover, this initial testing revealed that the gel remained equally stable at both room temperature and at 37C and that its mechanical properties did not change measurably. For future testing, we would propose to elevate the temperature of the gel further until there became perceptible differences so that an upper temperature limit could be established.

4. PROPOSED FUTURE DIRECTION

The current pullulan-IONP gel formulation will be examined *in vivo* in a tumor bed and exposed to hyperthermia therapy. To test the efficacy of this treatment, we plan to grow orthotopic tumors in nude mice, and treat the tumors through resection. We then plan to

spread the gel onto the tumor cavity and expose the mice to an AMF. We will measure the heating of the tumor bed tissue and assess these mice as well as a control group for signs of tumor regrowth. We will eventually sacrifice the mice and test IONP cell uptake kinetics via histology and TEM analysis.

The next important step in this technology is to move it into another realm of application: targeted IONP. In order for IONPs to be a maximally effective therapy, they must be specifically delivered to tumors and targeted into tumor cells (see Figure 5 below). Currently, research is being done to conjugate IONPs with an antibody targeted to a tumor marker. These targeted IONP can be delivered systemically through the circulation and are preferentially taken up by cancer cells expressing the tumor marker. While systemic administration of targeted IONP may be the only solution for treating metastases, the current targeted IONP techniques need improvement to achieve adequate IONP accumulation in tumor cells. A biocompatible tumor cell-targeted IONP gel in a surgical bed would improve the therapeutic efficacy of hyperthermia by localizing the IONP and hence the heating in the tumor cells and sparing normal cells.

Another realm of further development is incubated uptake within the body. Ultimately, a gel that could be coated via laparoscopic techniques onto tumors and incubated for a period of time to secrete IONPs into the cancer cells would maximize the therapeutic ratio for this treatment. The mechanical properties of gels in general would be ideal for concentrating the IONPs into the tumor and reducing the amount of IONPs taken into healthy tissue or into other organs.

5. COMMERCIALIZATION POTENTIAL

There is great commercialization potential for an IONP biodegradable gel. Given this, we have a patent pending on this technology. As we have mentioned previously, remaining tumor cells post-resection remain a problem in cancer surgery. Not only does this gel have great upside for topical application, but also it could become an ideal cancer therapeutic for targeted and incubated uptake given further research. In summary, from our initial studies of this IONP pullulan gel, we have found it to be ideal for a high therapeutic ratio hyperthermia therapy.

ACKNOWLEDGEMENTS

Supported by the Dartmouth Center for Cancer Nanotechnology Excellence: NCI-CCNE U54CA151662-03.

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Figure 1.
An example of IONPs being injected onto the surface of a tumor on the lower jaw; the diffuse nature of the tumor prevents the IONPs from remaining localized on the tumor.

Figure 2.
Xanthan gum IONP gel: mechanical property testing. Concentration: 0.98 mg/mL

Figure 3.
(from top to bottom): 20 nm nanomag-D SPIO Dextran 108 mg/mL pullulan hydrogel, 80 nm BNF Dextran 38 mg/mL pullulan hydrogel, 100 nm nanomag-D SPIO Dextran 10mg/mL pullulan hydrogel.

Figure 4.
(from left to right): After 1 hour incubation at 37C with 10 mL H₂O – biodegradability of 20 nm nanomag-D SPIO Dextran 108 mg/mL pullulan hydrogel, 80 nm BNF Dextran 38 mg/mL pullulan hydrogel, 100 nm nanomag-D SPIO Dextran 10mg/mL pullulan hydrogel.

Figure 5.
Example of targeted uptake (Nano Life, Giustini et al, 2010)⁶