Published in final edited form as:

Biomaterials. 2015 August ; 61: 299–306. doi:10.1016/j.biomaterials.2015.05.010.

Poly(lactide-co-glycolide) Microspheres for MRI-Monitored Delivery of Sorafenib in a Rabbit VX2 model

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Abstract

Transcatheter arterial embolization and chemoembolization are standard locoregional therapies for hepatocellular carcinoma (HCC). However, these can result in tumor hypoxia, thus promoting tumor angiogenesis. The anti-angiogenic agent sorafenib is hypothesized to improve outcomes; however, oral administration limits patient tolerance. Therefore, the purpose of this study was to fabricate poly(lactide-co-glycolide) microspheres for local sorafenib delivery to tumors during liver-directed embolotherapies. Iron oxide nanoparticles (IONP) were co-encapsulated for magnetic resonance imaging (MRI) of microsphere delivery. Microspheres were fabricated using a double emulsion/solvent evaporation method and characterized for size, sorafenib and IONP content, and MRI properties. MRI was performed before and after intra-arterial microsphere infusions in a rabbit VX2 liver tumor model. The microspheres were 13 microns in diameter with 8.8% and 0.89% (w/w) sorafenib and IONP, respectively. 21% and 28% of the loaded sorafenib and IONP, respectively, released within 72 hours. Rabbit VX2 studies demonstrated that sorafenib microspheres normalized VEGFR 2 activity and decreased microvessel density. Quantitative MRI

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enabled *in vivo* visualization of intra-hepatic microsphere distributions. These methods should avoid systemic toxicities, with MRI permitting follow-up confirmation of microsphere delivery to the targeted liver tumors.

Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer and globally HCC is the 6th most common cancer [1–3]. Many patients with unresectable HCC undergo transarterial embolization (TAE) or chemoembolization (TACE) procedures wherein a catheter is placed in the femoral artery of the patient and guided selectively to tumor feeding arteries in the liver. Once the catheter is optimally placed, embolic and/or chemotherapeutic agents are co-delivered through the catheter locally to the tumors [4–6]. This allows for specific targeting of tumors and containment of the chemotherapy while also starving the tumor of its blood supply.

Conventional TACE typically involves delivering a mixture of the chemotherapy in Lipiodol® (ethiodized poppyseed oil) with follow-up infusion of embolic particles in an effort to locally contain the chemotherapy by avoiding perfusion-mediated wash-out [4–7]. However, recent studies have shown that after TACE procedures, the creation of ischemic conditions at the tumor promotes a pro-angiogenic response in order to allow the tumor to continue to thrive. This is evidenced by a prominent increase in patient serum VEGF levels within the first 24 hours with VEGF levels remaining elevated for up to one month after TACE procedures [8, 9]. This observed phenomenon is correlated to poor patient outcomes. As a result, with the approval of sorafenib (a multikinase inhibitor that targets VEGFR [10, 11] for HCC in 2007, several clinical trials have investigated the efficacy of combining oral systemic administration of sorafenib with TACE in order to address the observed pro-angiogenic response [12, 13].

Unfortunately, the systemic distribution of sorafenib is associated with potentially severe side effects such as gastrointestinal symptoms, hand and foot syndrome, and hypertension [14–17]. Clinical studies have indicated adverse events leading to requisite dose reductions in roughly 30% of patients [14, 18, 19]. Patient tolerance can be severely limited, which in turn limits permitted dose and associated patient response. Local delivery of sorafenib as part of a TACE procedure should be advantageous to locally address the tumor's pro-angiogenic response while improving patient tolerance.

Sorafenib is a hydrophobic drug, thus it is difficult to load sorafenib into pre-existing microsphere platforms such as commercially available hydrogel DC Bead®. In order to formulate sorafenib for TACE procedures, a novel platform is needed. Poly(lactide-co-glycolide) (PLG) is a biodegradable polymer already used in FDA approved devices such as Lupron Depot® and in POLYSORB™ sutures. Previous studies have validated the potential to fabricate PLG microspheres for TACE procedures [20–22]. The advantageous drug loading mechanism for PLG platforms involves encapsulating the drug during the fabrication process [23]. PLG microspheres can be loaded with either hydrophobic or hydrophilic drugs, and combined with its biocompatibility [24] can be a platform for translational applications in clinical settings.

The purpose of this study was to develop PLG microspheres that can co-encapsulate sorafenib and iron oxide nanoparticles to enable MRI-monitored local delivery of sorafenib to limit pro-angiogenic responses in liver tumors following transcatheter embolotherapies. After fabrication, the microspheres were characterized for size, loading and release properties before investigating MRI properties *in vitro*. After these initial *in vitro* characterization studies, a rabbit VX2 liver tumor model was employed to enable *in vivo* studies a) validating the potential for MRI-monitored tumor-targeted transcatheter delivery and b) investigating the elicited tumor responses.

MATERIALS AND METHODS

Materials

75:25 Poly (D,L-lactide-*co*-glycolide) (PLG RESOMER® RG 752H, MW=4000–15000) polymer was purchased from Sigma Aldrich (St. Louis, MO). Sorafenib tosylate was purchased from LC Laboratories (Woburn, MA). The iron oxide was in a ferrofluid solution (EMG 304) and was purchased from Ferrotec (Santa Clara, CA).

Microsphere Fabrication

Microspheres were fabricated via a double emulsion/solvent evaporation method. Specifically, 714 mg of PLG was dissolved in 1.71 mL dichloromethane and added to 71.4 mgs of sorafenib in 1.14 mL of DMSO to compose the oil phase. The water phase consisted of a 0.14 mL water-based suspension containing 142 mg of the iron oxide. The oil phase and water phase were combined and homogenized at 7000 rpm for 30 seconds before adding 15 mL of 1% polyvinyl alcohol. Afterwards, the solution was homogenized again at 7000 rpm for 2 min. and poured into a stirred beaker containing 240 mL 0.5% polyvinyl alcohol. The microspheres were stirred in the beaker at least three hours for solvent evaporation before these were collected, washed and lyophilized.

Microsphere Size and Morphology Characterization

Microspheres were imaged for morphology using a Leica DM IL microscope with a Leica DFC290 Camera (Leica Microsystems, Wetzlar, Germany). Microsphere diameters were determined via Image J software analysis of the resulting images.

Sorafenib and Iron Oxide Loading

To characterize the amount of sorafenib contained within the microspheres, the microspheres were dissolved in a solution of 60:40 Acetonitrile: 20mM Ammonium Acetate containing 1% DMSO at a concentration of 1 mg/mL. The samples were then spun down for 5 min. at 5000 rpm to separate the sorafenib from the PLG and iron oxide. The supernatant was then analyzed via high performance liquid chromatography (HPLC) using an Agilent 1260 Infinity Quaternary LC HPLC (Santa Clara, CA) system and Zorbax C18 column using the methods as described in Blanchet et al. [25]. HPLC determined sorafenib concentrations were used to estimate the weight percentages of sorafenib in the microspheres by simply dividing the sorafenib concentration by the overall microsphere concentration in the solution analyzed via HPLC. Similar calculations were then used to determine the percentage of sorafenib originally used in fabrication that was actually

retained post-fabrication (aka loading efficiency) by dividing the weight percentage by weight percentage of sorafenib used during fabrication. Similar procedures were performed for characterization of iron oxide content within the microspheres. In triplicate, microspheres were dissolved in nitric acid and then prepared for inductively coupled plasma mass spectrometry (ICP-MS) in 2% nitric acid buffer solutions containing 5 ppb Yttrium as an internal standard. Calibration standards were prepared with concentrations of iron ranging from 0–100 ppb in 2% nitric acid buffer solution also containing 5 ppb Yttrium. ICP-MS was then performed to determine iron concentrations within these microsphere solutions. Similar to sorafenib measurements, weight percentages of iron oxide in the microspheres were determined as the ratio between the mass of iron oxide present in the prepared solutions measured via ICP-MS and the original mass of microspheres dissolved in the nitric acid. This weight percentage was then divided by the original weight of iron oxide used during microsphere fabrication to determine the percentage of iron oxide that was retained within the microspheres post-fabrication (aka loading efficiency).

Sorafenib and Iron Oxide Release

To study the release kinetics of the microspheres *in vitro*, in triplicate, 5 mg of microspheres were dispersed in 50 mLs of 1% sodium dodecyl sulfate in phosphate buffered saline. This solution was then placed in an incubator maintained at 37°C and at fixed time points 1 mL aliquots were withdrawn and filtered to separate out any microspheres that may have been withdrawn. Any microspheres withdrawn were returned and the 1 mL volume removed was replaced with 1 mL of fresh media to maintain a concentration gradient. The aliquots were then analyzed for sorafenib and iron oxide concentrations via HPLC and the QuantiChrom Iron Assay Kit (BioAssay Systems, Hayward, CA, USA).

MRI Phantom Studies

To characterize the T_2^* relaxivity properties of the microspheres, phantoms were created with microspheres embedded in 1% agar gels at concentrations ranging from 0–2 mg/mL. These agar gel phantoms were then imaged using a 7 Tesla Bruker Clinscan (Bruker, Billerica, MA, USA) with T_2^* mapping protocol. Specifically a GRE sequence (TR=600 ms, 1.5 mm slice thickness, 8 TE ranging from 4–25 ms) was used for T_2^* measurements. Image processing via MATLAB was performed to extract R_2^* relaxation values by fitting TE-dependent signal decay curves to an exponential function (data points at TE with relative SNR <10 were excluded to avoid noise bias). Finally, the microsphere concentrations were plotted against R_2^* values and linear trend line used to determine relaxivity (relationship between R_2^* and microsphere concentration).

Creation of Animal Model

Animal studies were performed under approval of Institutional Animal Care and Use Committee (IACUC). Two VX2 tumors were cultured in the hind limbs of New Zealand White rabbits and subsequently harvested when the tumors reached approximately 3 cm in diameter. Once harvested, the tumors were prepared to propagate the cell line and small fragments were sectioned for direct injections via a biopsy needle into the livers of additional New Zealand White rabbits (N=20) under ultrasound guidance (M7, Mindray

Medical Intl. Ltd., Shenzhen, China). Follow-up MRI was performed roughly 10 days post-liver implantation using a 7 Tesla MRI scanner to monitor tumor progression.

Rabbit Hepatic Artery Catheterization

Approximately two weeks post-implantation, once the tumors reached approximately 1 cm in diameter, x-ray digital subtraction angiography (x-ray DSA) (GE OEC 9800 Plus, GE Medical Systems, Salt Lake City, UT) was performed to selectively place a catheter into tumor feeding branches of the hepatic artery. After catheter placement, 50 mg of microspheres were infused. After the microsphere infusion, the catheter was withdrawn and the animal was immediately moved to the 7T MRI scanner for post-procedural imaging. To determine the exact dose of microspheres that was effectively delivered through the catheter, the remaining microspheres that were left undelivered in the vial and in the syringe were saved and analyzed via HPLC, using the same protocols as described above.

Histology and Immunohistochemistry Analyses

After post-procedural imaging, rabbits were recovered and survived for 24 hours before they were sacrificed. Afterwards, sections of the liver, stomach and lung were harvested and preserved in formalin for subsequent paraffin embedding, Prussian blue staining (demarcating iron-oxide accumulation) and H&E histological staining (1 cross section taken through the center of each tumor) as well as anti-CD31 immunohistochemistry (IHC) (3 separate cross sections through each tumor, each section within roughly 4 mm of the geometric centroid of the lesion) for microvessel density measurements. The subsequent slides were then imaged using a TissueFAXS microscope (TissueGnostics GmbH, Vienna, Austria). Anti-CD31 IHC slides were analyzed using associated HistoQuest software (TissueGnostics GmbH) to determine percentage of areas positive for CD31 staining within the tumor tissues (serving as a quantitative measure of microvessel density).

Western Blot Analyses

After the rabbits were sacrificed, roughly 1/2 of the tumor tissue volume and a 1–2mm border including surrounding normal liver parenchyma were immediately harvested and frozen at -80°C before the remaining harvested tissue was preserved in formalin. This tissue was then processed for western blot analyses for VEGFR 2 (Abcam Inc., Cambridge, MA, USA).

Quantitative MRI of Microsphere Delivery

Pre and post-procedural T_2^* maps were constructed from the respective MRI images using MATLAB; these tumor regions-of-interest (ROI) were drawn within these T_2^* maps to measure changes in T_2^* values before and after microsphere infusion.

Statistical Analysis

Results are presented as mean $\pm$ standard deviation unless otherwise indicated. Statistical analyses were performed via Stata (StataCorp LP, College Station, TX) using one way ANOVA analyses with Scheffe post-hoc corrections as well as unpaired Student's T-tests. Results with $p < 0.05$ were considered statistically significant.

RESULTS

Microsphere Characterization Studies

The fabricated microspheres were found to be spherical in shape and had an average size of 13 μm , however, the microsphere diameters ranged broadly from 2.5–65 μm (Fig. 1a,b). The weight percentage of sorafenib in the microspheres was $8.8\pm0.2\%$ which corresponds to a loading efficiency of 87.7%. The weight percentage of iron oxide in the microspheres was $0.89\pm0.10\%$ which corresponds to a loading efficiency of 4.5%. Sorafenib and iron oxide release studies indicated that 21% and 28% of the loaded sorafenib and iron oxide was released within 72 hours, respectively (Fig. 1c). *In vitro* agar phantom studies indicated that the microspheres produced significant negative contrast effects (signal reductions within T_2^* -weighted MR images) with T_2^* values decreasing from 111.1 ms to 33.3 ms with microsphere concentrations increasing from 0 to 2 mg/mL (Fig. 2).

Catheterization and MRI

Successful tumor inoculation occurred in 18/20 rabbits according to follow-up MRI; however, the 2 rabbits that did not show tumors upon imaging presented with small liver tumors upon follow-up necropsy. Of the remaining 18 rabbits, 6 rabbits were successfully catheterized for infusion of PLG sorafenib iron oxide microspheres, 6 were successfully catheterized for infusion of iron oxide-only PLG microspheres (thus serving as bland embolization controls), and the final 6 rabbits were left as untreated controls (no DSA performed). During the catheterization procedures, the residual dose of sorafenib left undelivered (i.e. remaining with dose vial) as determined by HPLC was 2.65 ± 0.80 mg or $5.3\pm1.6\%$.

Pre- and post-procedural MRI was performed and images processed with MATLAB to quantify differences in tumor R_2^* values before and after microsphere infusions (Fig. 3). Pre-procedural R_2^* values were 0.075 ± 0.028 ms with post-procedural R_2^* values of 0.105 ± 0.026 ms. The overall R_2^* between pre-procedural and post-procedural MRI images for individual tumors was 0.030 ± 0.018 ms.

Histology and Immunohistochemistry

Prussian blue histology indicated microsphere deposition primarily at tumor peripheries with limited non-targeted delivery to the stomach and no observed shunting to the lungs (Fig. 4).

Analysis of the rabbit anti-CD31 immunohistochemistry for microvessel density using the HistoQuest software indicated that sorafenib iron oxide microsphere treated tumors showed a $2.51\pm2.49\%$ area of positive staining, while iron oxide-only bland embolization treated tumors showed an $8.28\pm4.67\%$ area of positive staining and untreated control tumor samples indicated a $3.19\pm1.87\%$ area of positive staining (Fig. 5). Statistical analysis via one way ANOVA with a Scheffe post-hoc correction and Student's T-tests indicated statistically significant differences when comparing rabbit anti-CD31 IHC staining between tumors in sorafenib microsphere treatment groups and iron oxide-only bland embolization treatment groups ($p<0.01$). Rabbit anti-CD31 IHC staining for untreated control tumor specimens was shown to be significantly different from tumors treated with iron oxide-only microspheres

(bland embolization)($p < 0.01$). Conversely, embolotherapy with transcatheter infusion of the sorafenib-eluting microspheres produced no significant differences in tumor anti-CD31 staining when compared to anti-CD31 staining in untreated controls ($p > 0.27$).

Western Blot Analyses

Western blot analyses showed that untreated control tumors demonstrated the least VEGFR expression (anticipated to be due to a lack of blood vessel embolization for induction of hypoxic conditions). VEGFR expression for sorafenib treated groups was 18.1% higher than that of the untreated controls, however VEGFR expression was still 13.2% less than VEGFR expression observed for iron oxide-only microsphere treated tumors (bland embolization) (Fig. 6).

DISCUSSION

Recent studies have hypothesized that the addition of orally administered sorafenib may improve patient outcomes following TACE procedures by addressing the observed pro-angiogenic response in the tumor. However, systemic administration and the associated side effects limits patient tolerance. Reformulation of sorafenib for local delivery during TACE procedures should increase dose delivered to the tumor for maximum therapeutic effect while also limiting systemic exposure, thus improving patient tolerance. Furthermore, we included iron oxide nanoparticles within our formulation to allow clinicians to employ post-procedural MRI to visualize microsphere delivery to the targeted tumor(s). In cases wherein a tumor is supplied by multiple branches of the hepatic artery, infusion from a sub-optimal catheter position could result in portions of the tumor left untreated. Imaging of microsphere delivery would allow physicians to re-treat patients from an alternative catheter position if necessary. These follow-up measurement may also prompt adoption of alternative treatment modalities should sub-optimal delivery predict poor long-term outcomes. Our studies were therefore focused upon the fabrication of PLG sorafenib iron oxide microspheres for selective sorafenib delivery during transcatheter embolotherapies (TAE/TACE) and validation of the potential efficacy of these microspheres in the rabbit VX2 liver tumor model. In our studies, we found that local delivery of the developed PLG sorafenib iron oxide microspheres enabled site-selective MRI-monitored delivery to liver tumors. Additionally, the studies showed that delivery of the PLG sorafenib iron oxide microspheres normalized tumor VEGFR protein expression after the procedure. This finding suggests that PLG sorafenib microspheres may be an advantageous in the setting of transcatheter embolotherapies as these may address the observed pro-angiogenic response.

The microspheres were fabricated via a double emulsion/solvent evaporation method to achieve microspheres of sizes large enough for embolic purposes in a large mammal animal model. The fabrication method resulted in poly-disperse microspheres spanning 2.5–65 micron in diameter. This could potentially be advantageous for embolization of vessels with broadly varying sizes; however, if improved monodispersity is desired, microsphere filtration and screening can be performed, or alternative methods such as microfluidics or spray drying employed to improve monodispersity of the fabricated microspheres. Furthermore, we did not observe microsphere passage through the capillaries to veins that

would ultimately lead to microsphere deposition in the lungs, despite the fact that we did not fractionate our microspheres before microsphere infusion. While smaller microspheres used clinically are known to have higher risk of ultimately depositing in the lung, we did not observe this in our study. This may be due to use of the rabbit model and potentially smaller capillary sizes. Additionally, this could be attributed to the fact that only a limited number of lung sections were studied during our analyses. Future studies focused on fabricating larger microspheres for clinical translation may be necessary.

Sorafenib's hydrophobicity does not enable it to be loaded on to the DC Bead® via the reversible ion exchange mechanism without chemical modification that may affect potency. Additional prior research has investigated dispersing sorafenib in a viscous iodinated oil called Lipiodol® [26, 27]; these efforts have been promising, future comparisons between Lipiodol® and microsphere-based delivery platforms will be important to compare resulting anti-tumor efficacy and systemic toxicities [28].

Wu and Wang [29] have rigorously characterized PLG degradation. The degradation occurs in four stages: hydration, initial degradation, further degradation and solubilization. Payload release from the microspheres occurs as the PLG undergoes degradation and the sorafenib and iron oxide are able to be carried out of the microspheres by the release media that penetrates into the microspheres. A similar four-stage process is expected for PLG degradation and subsequent sorafenib and iron oxide release in both *in vitro* and *in vivo* settings [29]. Given that the last stage of PLG degradation is solubilization to lactic acid and glycolic acid, it is expected that a final small burst release of the sorafenib and iron oxide should occur during last degradation stage. Given that both sorafenib and the iron oxide contrast agent are released upon degradation, it may be possible to infer the kinetics of sorafenib release based upon *in vivo* observations of MRI signal changes; however, further studies would be required to validate potential efficacy of latter approach. These PLG microsphere were anticipated to completely degrade over the time course of *in vivo* infusion and drug release. Microsphere degradation may be advantageous to a) avoid extended periods of tumor hypoxia post-embolization thus further mitigating angiogenesis or to b) permit retreatment by maintain vessel patency. Further studies are clearly warranted to validate these additional potential benefits to our approach.

During TACE, the creation of hypoxic conditions at the tumor, caused by blood vessel embolization, results in a sharp increase in VEGF levels within 24 hours thus promoting angiogenesis [8, 9]. The latter condition prompted the selection of a 24-hour survival time point for our current study. The observed normalization of VEGFR expression after PLG sorafenib-eluting microsphere infusion, when compared to iron oxide-only microsphere infusions (bland embolization controls), suggests that our developed drug delivery platform can modulate the associated increases in VEGF during transcatheter embolotherapies (TAE/TACE).

Our western blot data supported the observed anti-CD31 microvessel density measurements. Iron oxide-only microsphere treated tumors demonstrated the highest microvessel density, potentially due to vessel embolization, associated hypoxia and the subsequent promoting of elevated VEGF levels driving tumor angiogenesis. Tumors treated with sorafenib-eluting

PLG microspheres demonstrated a lower level of microvessel density, suggesting that sorafenib targeting of VEGFR reduced angiogenesis. The sorafenib microsphere treated tumor microvessel density was even reduced compared to levels in untreated controls. While producing rapidly growing lesions, (liver tumors reach 1 cm in diameter in 2 weeks), the VX2 cell line is typically hypervascular only at the rim of the tumor. During current study, we typically observed reductions in microvessel density at the tumor rim (Fig. 5) suggesting responses to the delivered sorafenib therapy as opposed to natural variations between tumor samples.

Quantitative MRI enabled us to properly evaluate changes in tumor T_2^* values in order to confirm that a dose of microspheres was delivered to the liver tumors, thus confirming success of the catheterization/infusion procedures. If translated, this would enable clinicians to monitor and confirm delivery of the PLG sorafenib iron oxide microspheres. This would be advantageous over alternative embolic microsphere platforms currently in use, as no clinically available microspheres are labeled to provide contrast under any imaging modality. However, iron release may limit the time period over which the microspheres can be imaged following infusion. For our studies, we performed immediate post-procedural MRI following the catheterization procedures. In clinical settings, immediate follow-up imaging should be feasible with combined x-ray DSA / MRI procedure suites (CITE). Alternatively, patients could undergo follow-up MRI scan within separate imaging suite to depict microsphere biodistribution; given iron release characteristics, follow-up imaging would likely be required within a relatively short interval post-infusion (12–24 hrs). However, iron release from the microspheres may also be considered advantageous given that long-term persistence within the tumor could be problematic precluding use of conventional gadolinium-enhanced MRI for follow-up imaging of tumor response (signal drop-out due to iron persistence obscuring depiction of enhanced viable tumor volumes).

Finally, while we infused consistent microsphere dose volumes for each treatment group and embolic angiographic changes were observed post-infusion in each animal, we did not perform invasive secondary assessments of arterial blood flow changes and/or tissue hypoxia. Iron-oxide loaded PLG microspheres (without sorafenib) were used as a surrogate for the microspheres conventionally used for bland embolization procedures in clinical-settings; our rationale was that these permitted MRI confirmation of procedural success (delivery to the targeted tumors). However, additional comparison studies will be valuable to determine whether deposition of iron-oxide loaded PLG microspheres alone (independent of induced hypoxia effects) can induce the observed VEGF spikes.

CONCLUSION

PLG microspheres enabled selective MRI-monitored transcatheter delivery of sorafenib to liver tumors in the rabbit VX2 model eliciting reductions in both VEGFR levels and microvessel density within 24 hours of the infusion procedures. The developed microsphere platform also permitted quantitative MRI for confirmation of procedural success and proper catheter-selective tumor targeting. These studies suggest the potential to use PLG sorafenib-eluting microsphere to reduce angiogenic effects during catheter-directed embolotherapies while avoiding systemic sorafenib exposures for improved patient tolerance.

Acknowledgments

The authors would like to acknowledge Jodi Nicolai for her work in preparing the VX2 samples. We would also like to acknowledge the Center for Translational Imaging, Mouse Histology and Phenotyping Laboratory, Quantitative Bioelemental Imaging Center, Center for Advanced Microscopy, and the Simpson Querrey Institute Equipment Core Facility at our institution for their assistance and resources. Support for the research came from the National Cancer Institute, and the National Institute of Biomedical Imaging and Bioengineering (NIBIB) (01CA159178, R01CA141047, R21CA173491 and R21EB017986). Additional support came from the Society of Interventional Radiology Foundation (SIRF), and the American Cancer Society (ACS) – Illinois (ACS 279148).

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Figure 1.

a) Confocal microscope image of PLG sorafenib iron oxide microspheres at 200x magnification. Scale bar represents 50 μm . **b)** Size histogram of microsphere diameters. Average microsphere diameter was 13 μm . **c)** Sorafenib and iron oxide release from the microspheres.

Figure 2.

a) T₂* weighted MRI image of PLG sorafenib iron oxide agar phantoms. With increasing microsphere concentration, there were increased decay rates due to the signal dephasing effects of the iron oxide. **b)** Rates of signal decay increased with respect to increased microsphere concentration. **c)** R₂* values calculated from the signal decay curves in (b) plotted against microsphere concentration. Slope of linear fit line provides R₂* relaxivity estimation.

Figure 3.

a) Pre-procedural T_2^* weighted MRI image of the liver (surrounded by white arrows). Tumor position is indicated by red arrows. **b)** Post-procedural T_2^* weighted MRI image of the liver shows intense signal loss at the tumor due to the catheter-directed delivery of the iron oxide containing PLG microspheres. **c)** Reconstructed R_2^* map of the tumor superimposed upon corresponding pre-procedural T_2^* weighted image. Average tumor R_2^* value from the map was determined to be 0.038 ms^{-1} . **d)** Reconstructed R_2^* map of the tumor superimposed upon corresponding post-procedural T_2^* weighted image with a marked increase in overall tumor R_2^* . Average tumor R_2^* value from the map was determined to be 0.086 ms^{-1} , thus indicating an increase in tumor R_2^* value after microsphere delivery. Corresponding R_2^* map colorbars define R_2^* values from 0–500 ms^{-1} .

Figure 4.

a) Representative control tumor sample shows no positive Prussian blue staining as no microspheres were delivered. Scale bar represents 1 mm. **b)** PLG sorafenib iron oxide microsphere treated tumor tissue sample shows positive Prussian blue staining at the tumor periphery, thus confirming proper catheter-directed tumor targeting. Scale bar represents 500 μm. **c)** Prussian blue staining was also performed for stomach tissue as potential complications can occur with non-targeted delivery of the microspheres to the stomach. Minimal delivery of microspheres to stomach was observed. Scale bar represents 500 μm. **d)** Prussian blue staining was also performed for lung tissue as lung shunting could also result in complications. No microsphere deposition was observed in lung tissues. Scale bar represents 500 μm. Images were acquired at 200x using a TissueFAXS microscope (TissueGnostics GmbH, Vienna, Austria).

Figure 5.

Rabbit CD31 IHC slides were imaged at 200x using TissueFAXS microscope and analyzed using associated HistoQuest software to determine percent areas of positive brown staining within the tumor tissue to evaluate microvessel density. **a)** Sorafenib treated tumor tissue (expanded views of tumor ROIs from resected liver sections) demonstrated decreased areas of positive staining when compared to both iron-oxide only microsphere treated tumor tissues (**b**) as well as untreated control tumor tissues (**c**), thus indicative of a anti-angiogenic effects. Scale bars of zoomed in tumor images depict 500 μm.

Figure 6. VEGFR expression increases with embolization with iron oxide only microspheres, while the sorafenib treated tumors reduces VEGFR expression to baseline levels before embolization