Reproducible Noninvasive Characterization of *In Situ* Forming Implants Using Ultrasound

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Statement of Purpose: Treatment of solid tumors has traditionally been ineffective when drug is delivered systemically, while intratumoral delivery has significant benefits. With phase sensitive drug delivery systems, a drug depot can be delivered directly into the tumor via a simple injection [1]. The rate of phase inversion of these implants has been shown to be inversely related to the drug release rate [2]. Currently there is no existing system that facilitates the long term analysis of these phase inverting matrixes, while concurrently presenting a means of visualizing the process [3]. In this study the goal was to show that by combining diagnostic ultrasound (US) with image analysis techniques one can qualitatively and quantitatively monitor the long term phase inversion dynamics of the phase sensitive drug delivery system noninvasively.

Methods: Three different molecular weight poly (DL-lactide-co-glycolide) (PLGA) polymers were used for comparison in the study (Table 1).

50:50 PLGA	M_W (Da)	Inherent Viscosity
2A	15,000	0.16 dL/g
3A	29,000	0.28 dL/g
4A	64,000	0.46 dL/g

To obtain solvent release data and formation data simultaneously, implants were formed in 1% agarose phantoms by dropping a 40 wt% PLGA/NMP solution into the phantom while the agarose was liquid (N=4). The phantoms were then placed in 37°C ddH_2O and kept well mixed on an incubated shaker table. Release samples were taken every other hour for the first 10 hrs and then once a day for 5 days. US images were acquired at the same time as the NMP release samples (Fig 3). The transducer was kept in a fixed position and images were obtained through the bottom of the agarose phantoms. The images were analyzed using MATLAB to determine the formation rate of the constructs, swelling, and the change in gray-scale intensity over time. The region of interest (ROI) was selected from the original gray-scale image, then an intensity based segmentation method was employed. The region was filled to create a total area picture, which was also used to determine the swelling (Fig 1).

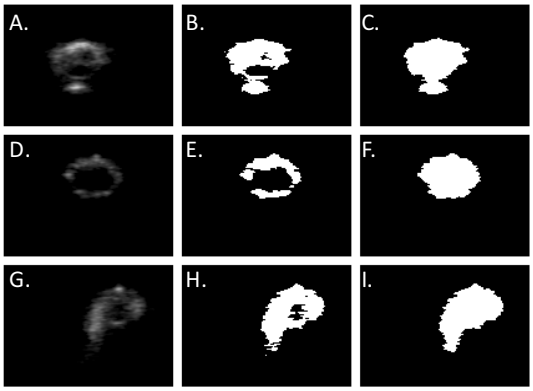

Fig 1 Gray-scale US images of 2A (**A-C**), 3A (**D-F**), and 4A (**G-I**) implants after 4 hrs in an agarose phantom.

To analyze the images, an optimal threshold value was found and applied (Fig 1B, 1E, 1H), and a total area image was generated by filling the interior region (Fig 1C, 1F, 1I). At each time point percent formation was determined by the ratio of the threshold image to the total area image.

Results/Discussion:

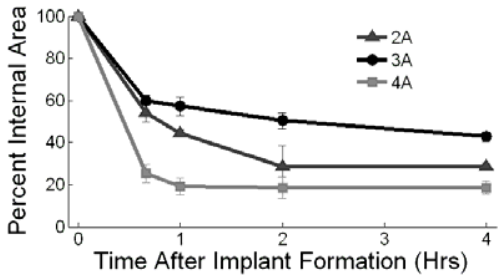

Fig 2 Quantitative formation data for three molecular weight polymer implants over the first 4hrs of formation

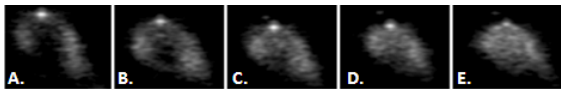

Fig 3 Representative US images of 4A implant after 10min (A), 8hr(B), 24hr(C), 48hr(D), and 120hr(E).

Our results showed that the 4A implant formed at a significantly faster rate than 2A and 3A implants (40%), reaching phase inversion of approximately 75% of the total cross-sectional area during the first 40min of formation. Although the 2A and 3A implants showed similar initial precipitation rate, at one hour the 2A rate of formation became faster (55%) than that of 3A (40%). The phase inversion began to slow significantly after 2hrs for all polymers (with the 2A, 3A and 4A precipitated polymer occupying approximately 70, 50 and 80% of the cross-sectional area, respectively) (Fig 2). Based on relevant literature, we anticipated a direct dependence of phase inversion on polymer molecular weight (due to the decreased solvent affinity with increase in molecular weight). However, our US data indicate that 2A precipitation occurred faster than that of 3A. The difference in the theorized and the actual formation process may be a result of increased osmotic drive contributed by polymer degradation products resulting in a higher H_2O concentration within the implant. The images also showed the biphasic nature of the implants during phase inversion (Fig 1and 3). Importantly, neither the difference in rate of phase inversion between 2A and 3A polymers nor the biphasic nature of the implant formation would have been possible to describe nondestructively using existing modalities for studying the dynamics of in situ implant formation.

Conclusions: Our study demonstrates the potential of noninvasive imaging in formulation and analysis of drug delivery systems. By combining nondestructive US imaging and quantitative image analysis, the formation process of phase inverting implants could be described in the same implant over time. The same technique can potentially be applied to any type of in situ forming implant aiding in development of future drug delivery systems. This work was supported by R01CA1118399 to AAE and TRN103514.

References:

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