Development of Tissue Equivalent Phantoms for Improved In Vitro Characterization of In Situ Forming Drug Delivery Implants

Monika Goss¹, Haoyan Zhou¹, and Agata Exner¹,²

Case Western Reserve University, Cleveland, Ohio, 44106, Country; Departments of Biomedical Engineering¹ and Radiology², Cleveland, Ohio, 44106, USA
monika.goss@case.edu

ABSTRACT SUMMARY

Various polycrylamide tissue equivalent phantoms were developed for use in characterization studies in order to obtain more realistic phase inversion, drug release, and degradation information for in situ forming implant (ISFI) formulations. Phantoms were prepared with consideration of modulus, water content, and swelling characteristics to better mimic potential injection tissue sites in vivo. Based on these parameters, a phantom with optimal characteristics can be selected for more representative in vitro implant assessment studies.

INTRODUCTION

There is a striking lack of correlation between in vitro and in vivo implant performance (including formation, drug release, and polymer degradation) of injectable, in situ forming implants which has impeded successes in the field¹. Previous studies in our laboratory showed that the environment immediately surrounding an implant significantly affects the implant’s drug release profile. For example, it was shown that an implant formed in vitro in PBS released 2 fold more drug than tumor tissue for poly(lactic-co-glycolic) acid implants formulated with NMP and releasing the model drug fluorescein². Prior research also demonstrated that tissue density and compliance may play a considerable role in the process of implant formation with the phase inversion rate in vivo being significantly faster than in vitro².

Accordingly, there is a clear need for improved in vitro paradigms that are more representative of the ultimate tissue site of action of the investigated ISFI formulations. This study aimed to develop tissue-equivalent phantoms that would represent a range of characteristics such as modulus and swelling / water uptake and can be used in future in vitro implant formation, drug release, and degradation/erosion studies.

EXPERIMENTAL METHODS

Phantom Preparation

Polyacrylamide phantoms were prepared in a range from 4% to 32% (w/v) using the chosen percentage of acrylamide and corresponding amount of phosphate buffered saline pH 7.4 (PBS). Free radical polymerization was performed using 10% ammonium persulfate (APS) and tetramethylethylenediamine (TEMED) as the initiator and catalyst, respectively. 0.05% titanium dioxide was added for use as a scattering agent in order to make the phantom echogenic.

Mechanical Testing

Samples to be used in mechanical testing studies were made following the above protocol. The solution was poured into a Teflon mold to polymerize, giving sample dimensions of 5 mm in diameter and 2.5 mm in height.

Unconfined compressive mechanical testing was performed on the acrylamide samples using a rheometer (Rheometrics, NJ). A strain rate of 0.01/s was used and the testing time was optimized for each sample in order to achieve at least 30% strain. The elastic modulus was then calculated using the linear range of the stress-strain curve generated by the rheometer. A first-order polynomial fit was used to determine the linear region using a cut-off coefficient of determination, R², of 0.98.

Water Uptake

Polyacrylamide samples were made as above and allowing the solution to polymerize in a 24-well tissue culture plate. Once set, samples were weighed to find the initial mass. Samples were then stored in PBS pH 7.4 in an incubator at 37°C. Samples were then removed daily and weighed to find the change in mass.

RESULTS AND DISCUSSION

A range of acrylamide samples tested resulted in an elastic modulus range from 4.39 ± 0.18 kPa to 638 ± 2 kPa. Adipose breast tissue is well mimicked by an acrylamide phantom of 7%, or about 20 kPa, liver tissue corresponds to 4% acrylamide, giving a
modulus of about 4 kPa, and muscle tissue corresponds to 6% acrylamide, or about 16 kPa\(^3\).

![Scattered Acrylamide Modulus](image1)

**Figure 1.** Modulus of acrylamide phantoms

![Acrylamide Swelling at 37C](image2)

**Figure 2.** Water uptake profile for various acrylamide percentages over time

Samples increased in weight up to 285% of their initial values. Acrylamide percentage was inversely proportional to swelling rate. All samples plateaued by day 7 and ceased to swell, suggesting that all phantoms should be maintained in a hydrated environment for one week prior to their application in implant studies.

As a demonstration of noninvasive nondestructive implant visualization using the developed phantoms, formulations consisting of PLGA dissolved in NMP were injected into voids formed in the 50 kPa acrylamide phantoms. The phantoms were then imaged with both B-mode clinical ultrasound and ultrasound elastography (Figure 3), which our group has utilized in prior studies to gain quantitative data on implant formation rate\(^4\), rate of drug release, and more recently polymer degradation (data not yet published).

![Day 1, Day 3, Day 5, Day 9, Day 14](image3)

**Figure 3.** Representative ultrasound (top) and ultrasound elastography (bottom) images of 50 kPa acrylamide phantom with embedded PLGA samples.

**CONCLUSION**

These results indicate that phantoms that accurately mimic the modulus and hydration properties of human tissue including adipose breast tissue, liver tissue, and muscle tissue are feasible. Such phantoms can become an integral tool in longitudinal characterization and prediction of implant performance *in vivo*.

**REFERENCES**


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