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Assessment of Imagining and Material Characteristics of a Radiopaque, Thermoresponsive Hydrogel for Intratumoral Administration to Solid Tumours

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Title: Assessment of imaging and material characteristics of a Radiopaque, Thermoresponsive Hydrogel for Intratumoral Administration to Solid Tumours

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Abstract

Introduction

Thermoresponsive hydrogels are gels which have different properties at varying temperatures. The objective of this study was to assess the material characteristics, imaging properties and chemotherapeutic drug release profile of a novel radiopaque thermoresponsive hydrogel, which is liquid at room temperature but solidifies at body temperature, to determine potential suitability for intratumoral delivery.

Materials and Methods

An iodinated radiopaque thermoresponsive hydrogel was formulated using iodixanol at a range of concentrations and assessed for sol-gel transition, radiopacity and imaging using CT and US. A lead formulation containing 9.22% w/w iodixanol was evaluated for injectability, disintegration and dual drug release of cisplatin and paclitaxel from the hydrogel formulation.

Results

Radiopacity of the hydrogel increased in a concentration dependent manner but higher concentrations of iodixanol adversely affected the sol-gel transition of the hydrogel, therefore 9.22%w/w iodixanol hydrogel was identified as the lead formulation. This formulation was readily visible on both CT and US. The formulation was hand-injectable through a range of clinically relevant devices, had a sustained disintegration profile for up to 28 days, and was able to deliver a sustained release of chemotherapeutic drug for up to 10 days.

Discussion

Favourable imaging and material characteristics of this thermoresponsive gel are demonstrated, suggesting potential interventional oncology applications for image-guided intratumoral delivery of sustained-release chemotherapy.

Keywords

Thermoresponsive hydrogel, iodixanol, imaging, radiopaque, injectable

Introduction

Systemic chemotherapy, in conjunction with radiation and surgery has long been the mainstay of cancer treatment. Lack of chemotherapy specificity, toxicity, off-target side effects and poor penetration into the tumour mass due to the altered tumour microenvironment limits efficacy, which has encouraged alternative delivery methods to be investigated (1-4). One such approach is direct injection of chemotherapeutics into the tumour, delivering chemotherapeutics or other ablative agents directly to achieve a high local concentrations while reducing systemic toxicity. *In vivo* animal studies have shown that direct intratumoral instillation of a chemotherapeutic solution can deliver more than 10-30 times intravenous doses, with minimal side effects (5). Intratumoral chemotherapy has also been explored clinically, with promising safety and proof of concept data generated (6-8). Direct intratumoral instillation is however limited by rapid solution clearance from the tumour site, resulting in inaccurate and unpredictable dosing. Therefore. attention has turned to the development of drug delivery systems which can facilitate delivery and retention at the site of action, and sustained intratumoral release of chemotherapeutics(4).

Thermoresponsive hydrogels offer the possibility of an injectable liquid at room temperature which can phase-transition to a solid hydrogel at physiological temperatures, and have been investigated in pre-clinical and clinical settings for intratumoral drug delivery in solid tumours (9, 10). This is an attractive drug delivery platform, due to the potential for image-guided administration without the need for surgical implantation of hardware. I*n-situ* gelation permits hydrogels to mould to the target tissue, facilitating a better fit than preformed implants (11), and facilitates sustained release of the drug at the required site of action (12-14). Hydrogels can also be disintegrable and the rate of disintegration can be tailored without the need for surgical removal.

Only a handful of thermoresponsive hydrogels have progressed to the clinical trials, to varying degrees of success (4, 15-19). This lack of clinical translation may be due to the fact that whilst the studied gelling properties are of importance, other factors which are of clinical relevance such as visibility using image-guidance, injectability, gel distribution, drug release, retention, and disintegration have not been given sufficient consideration during development. This study explores these clinically relevant factors of a novel thermoresponsive hydrogel formulation that has been specifically developed for intratumoral delivery.

Methods

Formulation of a radiopaque thermoresponsive hydrogel with and without chemotherapeutic drug loading

A proprietary thermoresponsive hydrogel was prepared with a commercially available iodinated contrast agent Visipaque® (iodixonal) 320mg I/ml. Iodixonal was added at concentrations of 0% w/w, 4.61% w/w, 9.22% w/w or 13.83% w/w, based on previous work carried out by Fatimi *et al.* (2016) (19). A dual drug-loaded version of this thermoresponsive hydrogel was prepared using two widely used chemotherapeutic agents, cisplatin and paclitaxel.

Assessment of material characteristics of iodixonal-labelled hydrogel formulation

The thermoresponsivity and shear-thinning properties of the hydrogel formulations were assessed using oscillatory and flow measurements, performed on a constant stress rheometer (AR-1000, TA instruments, Delaware, USA). The rheometer was equipped with cone/plate geometry (40mm diameter, 4^{o} cone angle). Degassed samples were dispensed onto the temperature controlled rheometer plate, pre-equilibrated to 20˚C, and covered with a solvent trap during testing. Oscillatory temperature sweeps from 20°C - 40°C were performed to determine sol-gel transition temperatures. Shear thinning properties of hydrogel formulations were performed at 20°C under increasing shear stress from 1-100 Pa. All experiments were repeated in triplicate. Data was processed using TA Data Analysis software.

Measurement of radiopacity of iodixonal-labelled hydrogel formulation

2ml of iodixonal labelled hydrogel formulation was added per well in a 12 well plate. Radiopacity was determined using a commercially available computed tomography (CT) machine (Ingenuity Core 128, Philips Healthcare, Best, Netherlands). Images were obtained using 0.8mm slice thickness, 0.4mm reconstruction interval, 168mAs and 100kV. A region of interest (100mm diameter) within each well was selected and the average density was calculated (minimum, maximum and standard deviation also recorded). All measurements were carried out in triplicate. Data shown is represented as the mean ± SEM.

Assessment of distribution of iodixonal-labelled hydrogel in ex vivo animal tissue

Ex vivo tissue (calves livers) was used to assess the distribution of injected radiopaque thermoresponsive gel. Prior to injection, the *ex vivo* tissue was heated to 37ºC in a water bath. Internal tissue temperature was recorded using a tissue thermometer. 5ml of hydrogel with 9.22% w/w iodixonal was injected into the *ex vivo* tissue model using an 18G 5cm vascular access needle (Cook Medical, IN, USA) at a constant rate to assess distribution of injected thermoresponsive gel in tissue. Three injections were made. Administration was monitored using a commercially available 12MHz linear ultrasound (US) probe (Xario, Toshiba) in grayscale B-mode. *Ex vivo* distribution post-injection was imaged using CT as outlined previously.

Assessment of injectability of hydrogel formulation

Uniaxial tensile testing was carried out to determine the force required to expel hydrogels from a syringe fitted with a needle or catheter, using a mechanical testing machine (Z050, Zwick/Roell, Ulm, Germany), fitted with a 5 kilanewton (kN) load cell (Fig.1). Samples were loaded into 2ml Leur-lok™ syringes and the appropriate medical device was attached to the syringe via Luer-lok™. A pre-load of 1N was applied and the end of test was determined to be the maximum extension (8.5mm); the distance equivalent to 0.5ml of gel (measured using Vernier's callipers). The speed of injection was defined as 2 ml/minute. The hydrogel solution samples were then loaded to failure and the solution expelled from the catheter was collected in a vial. All measurements were carried out in a minimum of triplicate. Results are represented as the mean ± SEM.

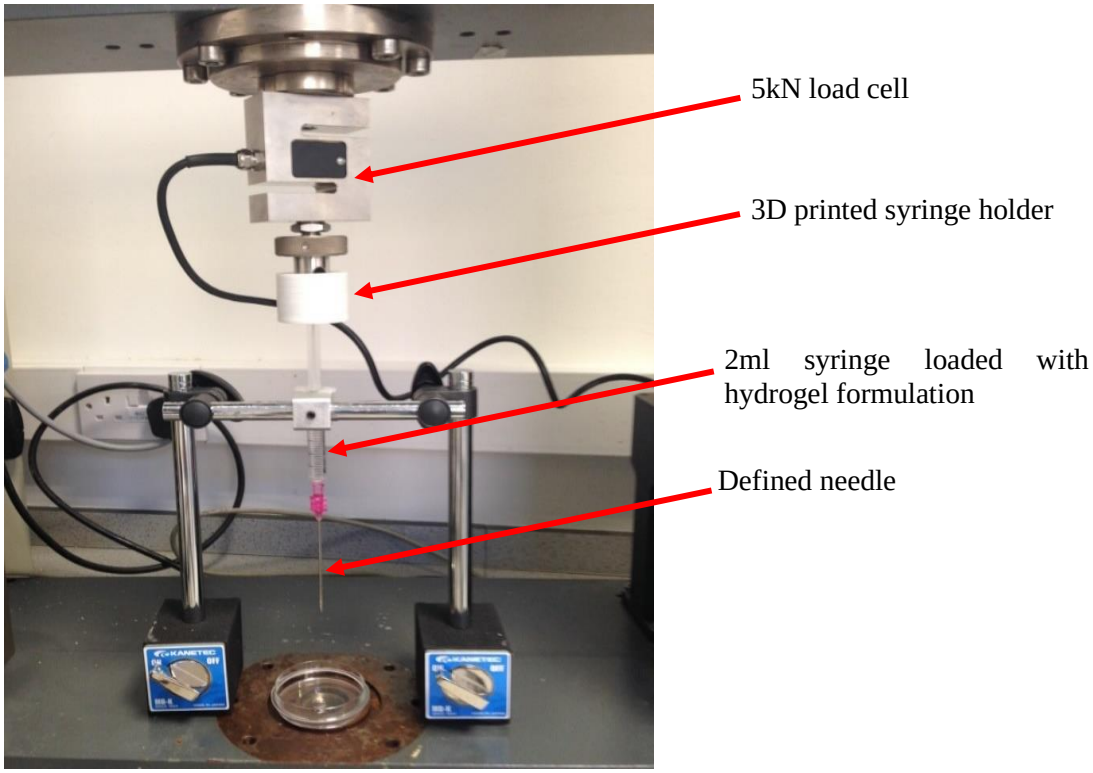


Fig 1. Representative image of injectability testing using a Zwick mechanical testing machine

Disintegration studies

1g of the gel formulation was added to a glass vial and submerged in a shaking water bath at 75 rpm, maintained at 37ºC, for thirty minutes to ensure complete gelation had taken place. 1ml of pre-warmed phosphate-buffered saline (PBS) (pH7.4) was added to the gel. At pre-determined timepoints (4h and day 1, 2, 3, 5, 7, 10, 14, 21 and 28), PBS was completely removed from the glass vial and the weight of the hydrogel + glass vial was recorded. After weighing 1ml of fresh pre-warmed PBS was added to the glass vial. All experiments were carried out in triplicate, for three independent batches. Results are represented as the mean of the three independent experiments ± standard error of the mean (SEM) (n=3).

Chemotherapeutic release studies

1 g of drug loaded hydrogel was added to a glass vial and submerged in a shaking water bath at 75 rpm, maintained at 37ºC, for thirty minutes to ensure complete gelation had taken place. 1 ml of pre-warmed PBS (pH 7.4) was added to the gelled hydrogel and at pre-determined timepoints (4h and day 1, 2, 3, 5, 7, 10, 14, 21 and 28) PBS was completely removed from the glass vial and then replaced with 1 ml of fresh pre-warmed PBS. Samples were transferred to a centrifuge tube and stored at -20°C until analysis. Analysis of released paclitaxel was performed using liquid chromatography (Agilent Technologies 1120 Compact LC) with a UV detector, based on a method by Kraitzer *et al.* (2008) (21). The mobile phase was Water: Acetonitrile (45:55), with a flow rate of 1ml/min, a run time of 10 min and detection at 227nm. Analysis of released cisplatin was performed using inductively coupled plasma mass spectrometry at a detection wavelength of 214.423, with a plasma flow of 15L/min, auxiliary flow 1.5L/min and nebulizer pressure of 200kPa. All experiments were carried out in triplicate, for three independent batches. Results are represented as the mean of the three independent experiments ± SEM (n=3).

Results

Impact of iodixonal on sol-gel transition of hydrogel formulations

Fig. 1(a) shows the effect of increasing concentrations of iodixonal on the material characteristics of the gel. The storage modulus (G') is the elastic component of the gel, which is a measure of structure within the gel. The sol-gel phase transition is marked by a abrupt increase in the G' of the gel, indicating a transition from liquid to semi-solid state at physiological temperatures as observed in Fig. 2(b). Concentrations of iodixonal up to 9.22% do not significantly impact on either the sol—gel transition temperature or the strength of the gel. However increasing the iodixonal concentration to 13.83% causes a significant reduction in sol-gel transition temperature and an increase in the strength of the gel.

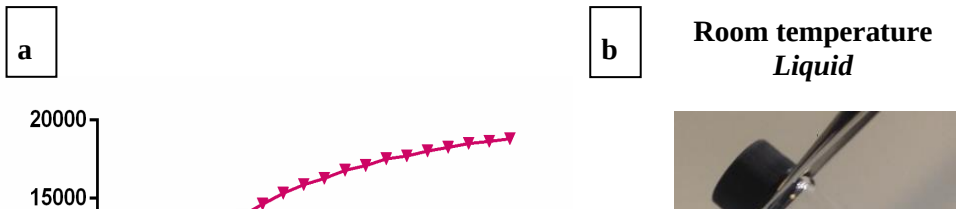
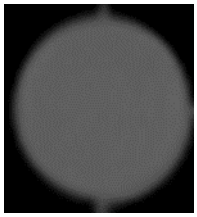
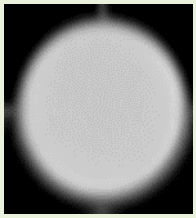
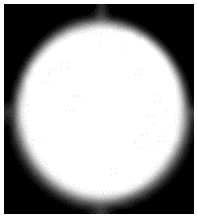


Fig 2. (a) Addition of iodixanol to the hydrogel formulation impacts the rheological properties of the thermoresponsive hydrogel in a concentration-dependant manner. Rheogram of the oscillatory temperature sweeps from 20°C - 40°C of hydrogel formulations containing different concentrations of iodixanol solution (b) Representative image of hydrogel formulation, at room temperature, in liquid state and following incubation at 37ºC, in gelled state. Hydrogel contains colouring for clarity.

Radiopacity of iodixonal labelled hydrogel formulations

The addition of iodixanol solution to the hydrogel formulation was seen to produce a homogenous gel at all concentrations evaluated, as evidenced by CT imaging (Table 1). Addition of iodixanol solution resulted in increased radiopacity in a concentration dependent manner. Gelation only minimally reduced radiopacity in all three iodixanol labelled hydrogel groups. Due to favourable rheological and radiopacity characteristics, the hydrogel containing 9.22% w/w iodixanol was selected for further assessment.

Table 1 Radiopacity of hydrogel formulation with increasing concentrations of iodixanol. Data shown is representative of the mean ± SEM (n=3). **HU=Hounsfield Units, w/w=weight by weight**

	Liquid (HU)	**Gelled (HU)**	**Coronal image of well plate on CT (window width 1488, level 346)**
9.22% w/w iodixanol solution	1523.7 ± 7.09	N/A	
Thermoresponsive hydrogel with 0% w/w iodixanol	33.57 ± 2.34	22.4 ± 2.06	
Thermoresponsive hydrogel with 4.61% w/w iodixanol	900.03 ± 5.15	697.11 ± 19.28	
Thermoresponsive hydrogel with 9.22% w/w iodixanol	1666.23 ± 6.98	1586.0 ± 12.03	
Thermoresponsive hydrogel with 13.83% w/w iodixanol	2428.83 ± 19.01	2378.7 ± 13.9	

Distribution of iodixonal-labelled hydrogel in *ex vivo* animal tissue

Injection into *ex vivo* calves tissue demonstrated that the radiopaque hydrogel (9.22%w/w iodixanol) was readily injectable, and moulded to the shape of the target tissue. On US, it was was found to be clearly visualised, anechoic and without speckling and it demonstrated satisfactory radiopacity on CT, where it was seen to both pool in parenchyma, and spread along vascular and biliary channels when punctured, before undergoing gelation and remaining localised (Fig. 3)

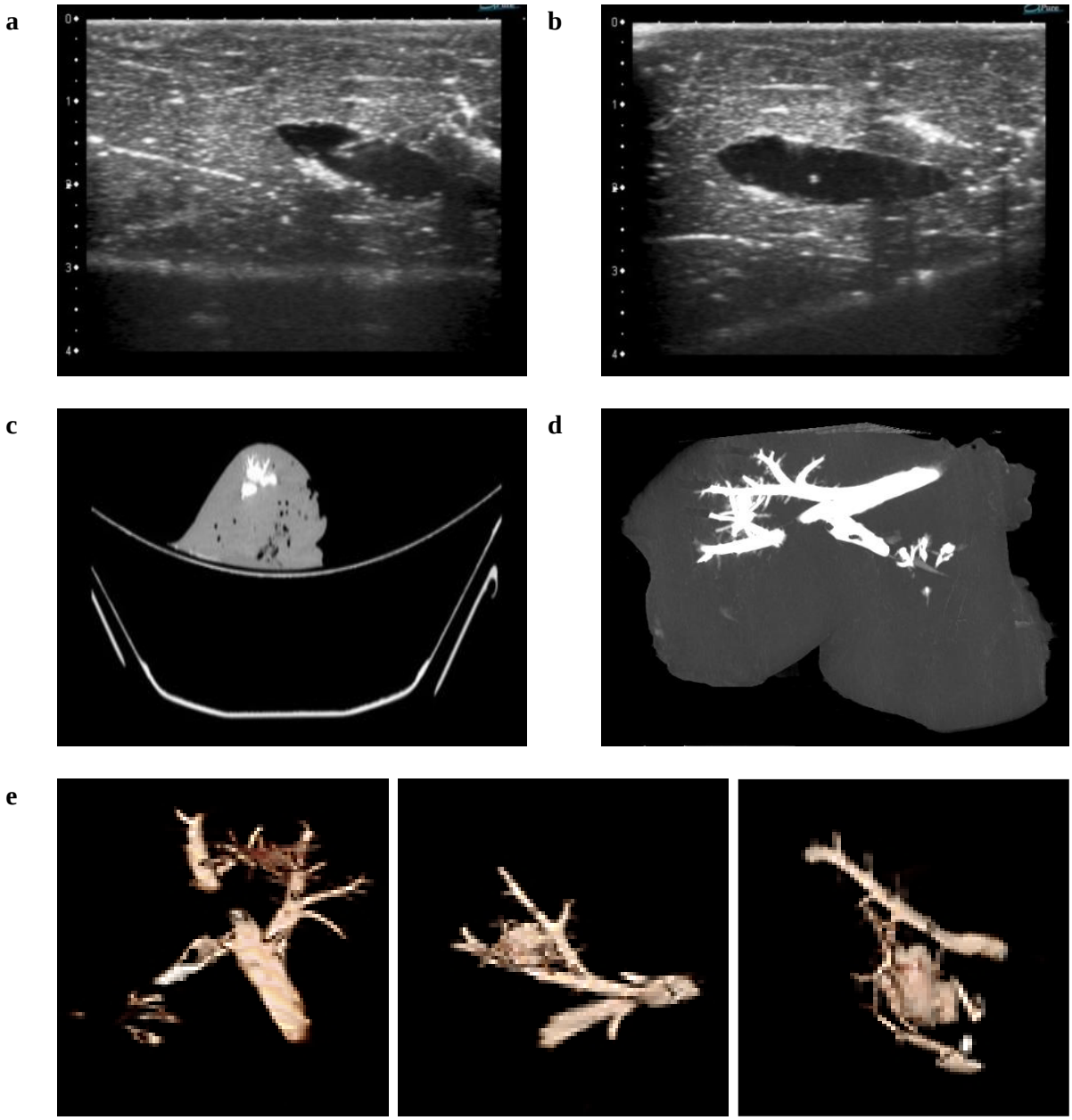
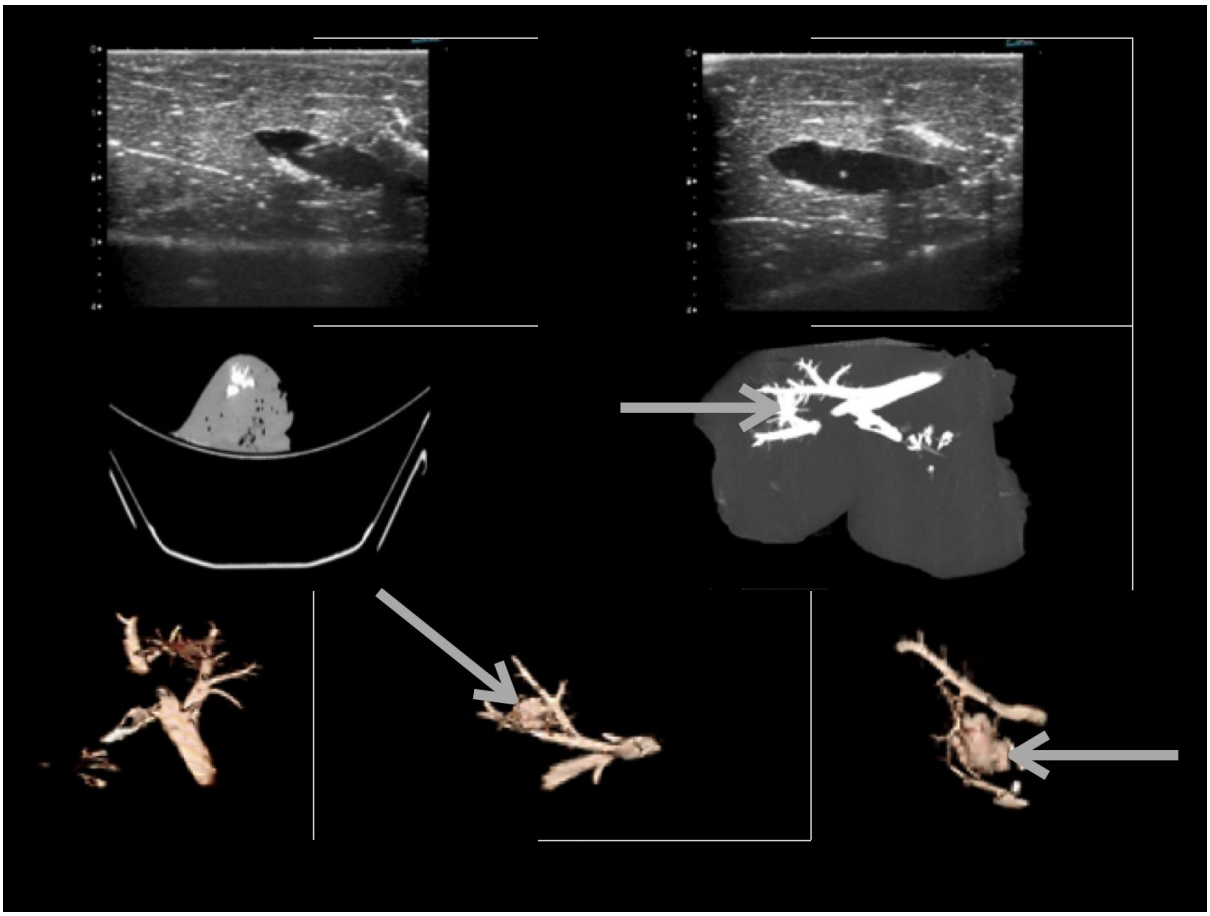


Fig. 3 *Ex vivo* tissue administration of radiopaque hydrogel can be visualised using CT and US. Representative (a&b) US and (c&d) CT images of radiopaque hydrogel distribution in *ex vivo* tissue.

Arrows indicate pooling of radiopaque hydrogel (d,e) CT images of radiopaque hydrogel spreading through vascular and biliary channels.



Injectability of hydrogel formulations

Rheological measurement of viscosity at 20°C showed shear thinning i.e. a decrease in viscosity with increasing shear which is beneficial for injectability. Radiopaque hydrogel was injectable across all needle gauges evaluated, with only the 23G device required a force greater than 50 N to achieve injection. Injection through a 65cm 5Fr catheter was also shown to be feasible. A force of 0 – 10 N was considered "easy to inject", and forces up to 50 N can be defined as "injectable" (20).

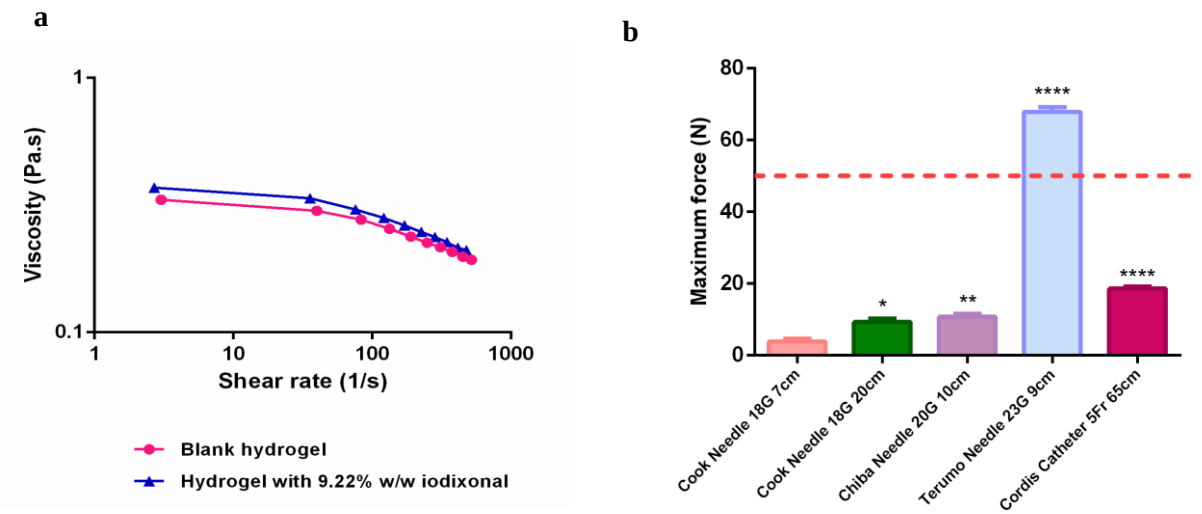


Fig. 4 (a) Blank and radiopaque hydrogel underwent shear thinning (a decrease in viscosity in response to increasing shear rate). Data shown is representative of the norm. (b) Injectability of 9.22%w/w iodixanol hydrogel formulation at 2ml/min. Increasing needle gauge and catheter length increases force required for injection. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ compared to Cook Needle 18G 7cm. Data shown is represented as the mean ±SEM (n= 3).

Disintegration and chemotherapeutic release from hydrogel formulations

Similar disintegration profiles for both the blank and drug-loaded radiopaque hydrogel were observed *in vitro* over 28 days (Fig. 5(a)), with both formulations demonstrating extended disintegration profiles. An initial decrease in mass was observed for both formulations after 4 h of exposure with a subsequent steady decrease in mass observed thereafter until 168 h, after which mass loss levelled off, with approx. 20% of the hydrogel still present at day 28. A slow disintegration of the gel is important to ensure it remains *in situ* for a sufficient period of time to enable the chemotherapeutic drug load to be released at the required site of action. *In vitro* release study of a dual drug-loaded hydrogel showed sustained release of cisplatin and paclitaxel for up to10 days. A significant burst release of both chemotherapeutic agents was observed in the first 24 h, with a higher burst and shorter sustained release of cisplatin. This is to be expected as cisplatin has significantly higher water solubility than paclitaxel and therefore will diffuse more rapidly into the surrounding aqueous medium driven by simple diffusion. Paclitaxel, with a lower water solubility, showed a smaller burst release and a more sustained release profile for up to to 10 days from the hydrogel. The rate of drug release from hydrogels has been attributed to chemical, physical and biological interactions between the hydrogel matrix, the drugs loaded and surrounding tissue (22).

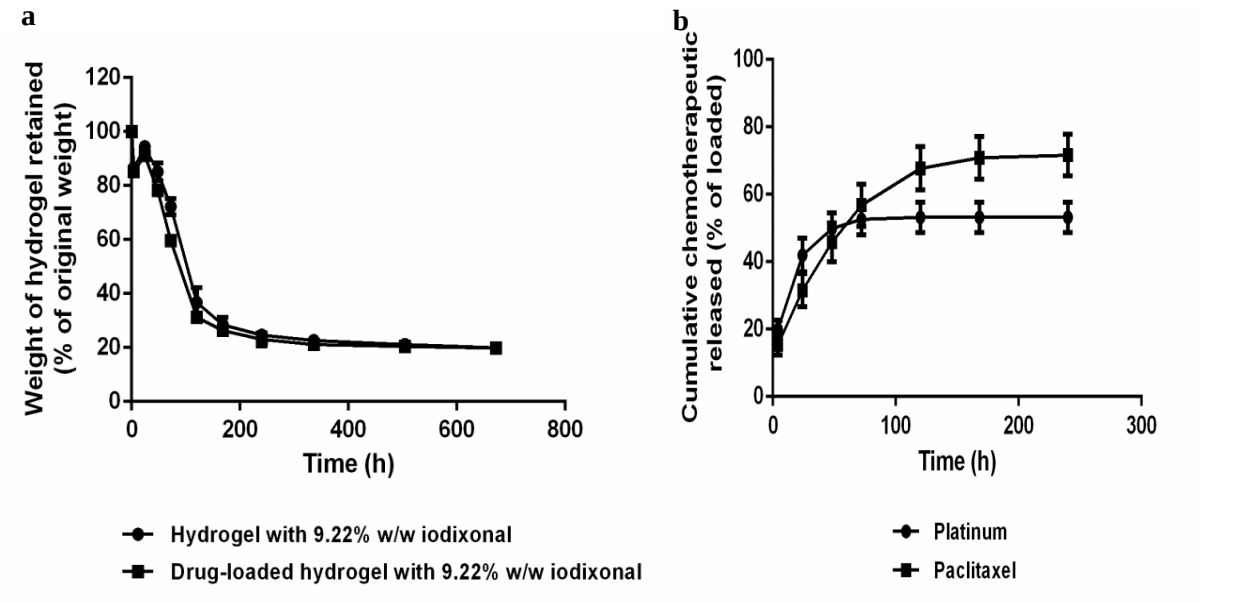


Fig. 5 (a) Disintegration of the blank and drug-loaded radioapaque hydrogel over 28 days. Disintegration determined by measuring loss in mass (% of original weight of hydrogel) per timepoint. (b) Cisplatin (measured as platinum) and Paclitaxel were released in a controlled manner from the drug loaded hydrogel over a 10 day period. Cumulative percentage of loaded cisplatin and paclitaxel released over a 10 day period (240 h). Data shown is representative of the mean of three independent experiments ± SEM (n=3).

Discussion

Thermoresponsive hydrogels can support the delivery of a range of chemotherapeutic agents, achieving high and lasting local concentrations (3, 23-25). To transition to such clinical use however, these gels must be both easily visible on imaging, and hand-injectable. This study demonstrates that combining an off-the-shelf iodinated contrast preparation with a novel thermoresponsive hydrogel formulation is feasible, and leads to a highly visible formulation both radiographically (on CT) and sonographically (on ultrasound). Furthermore, testing of viscosity and injectability demonstrates these formulations are injectable with forces that can be generated by hand. Disintegration studies suggest that prolonged retention within tissue is achievable and that sustained drug release of commonly used chemotherapeutic agents is possible and can be tuned dependent on the drug properties. These results suggest that the inherent properties of this thermoresponsive hydrogel are well suited to transition to the interventional radiology suite. This, combined with the potential for prolonged retention and the wide variety of chemotherapeutic agents which could be loaded into the hydrogel, offers exciting future treatment opportunities. This is particularly promising for infiltrative tumours which can be accessed, but are not amenable to ablation due to adjacent vital structures, such as in locally-advanced pancreatic cancer, cholangiocarcinoma, glioblastoma, and infiltrating mediastinal or peritoneal tumours.

In terms of clinical translation, further investigation is required to establish how thermoresponsive hydrogels behave on delivery into a variety of tissue types and tumour types. It will also be important to establish any toxicity associated with the use of the gel. Work is currently ongoing in a number of pre-clinical xenograft cancer models to establish efficacy of both the blank and chemotherapeutic loaded hydrogel and to determine whether there is any local or systemic toxicity associated with the hydrogel. The delivering proceduralist, likely an interventional radiologist, will also require an understanding of how these hydrogels behave in soft-tissue. In practice, injection force and spread

within various tumours and organs (and even organs in various states such as cirrhosis) can feel different. In this study, the investigators note that within calf liver, the gel pooled at the needle tip at low pressures, and flowed into vascular and biliary channels as injection pressure and volume increased. This would need to be validated in human tissue and for each clinical indication.

Conclusion

Thermoresponsive hydrogels can be designed to transform from liquid at room temperature to solid at body temperature. This study demonstrates that these gels can be loaded with various concentrations of iodinated contrast and chemotherapeutic agents while maintaining their inherent properties. Furthermore, the contrast-labelled thermoresponsive hydrogel is well seen on a variety of medical imaging modalities. The viscosity of the thermoresponsive hydrogels at room temperature permits hand-injection. The hydrogel shows a slow disintegration profile when held at body temperature. These characteristics suggest this novel thermoresponsive gel may have applications in interventional oncology, particularly in solid tumours accessible by image-guidance.

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