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Investigation of alginate gel inhomogeneity in simulated gastro-intestinal conditions using magnetic resonance imaging and transmission electron microscopy

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ABSTRACT

The inhomogeneity of alginate gel beads prepared by an external diffusion method has been characterised using spatially resolved nuclear magnetic resonance or "magnetic resonance imaging" (MRI) and transmission electron microscopy (TEM). The beads exhibited various degrees of inhomogeneity although reducing the length of exposure to the gelling bath and the presence of non-gelling ions decreased gel inhomogeneity. In order to gain information regarding the gastro-intestinal functionality of these beads for *in vivo* applications, they were exposed to simulated gastro-intestinal conditions. The increased polymer concentration at the edge of the beads was shown to persist throughout our gastro-intestinal model despite the centre of the bead becoming progressively more porous in nature. The porosity of the alginate gels has been quantified by image analysis of transmission electron micrographs and shown to depend on both location within the bead and gastro-intestinal conditions. We suggest that such changes in porosity of these alginate beads during simulated gastro-intestinal conditions may make these an attractive option for controlled delivery applications *in vivo*.

1. Introduction

Alginates are natural polysaccharides mainly isolated from various species of brown algae where their biological function is to provide both strength and flexibility to the cellular tissue. They are used in a wide range of applications particularly in the food and pharmaceutical industry due to their capacity to hold water, form gels and stabilise emulsions. Alginates are unbranched block co-polymers of (1 → 4)-linked residues of β-D-mannuronic (M) acid and α-L-guluronic (G) acid. The ion-binding properties of alginates are the basis for their gelling properties and have been the subject of much research interest (Emmerichs, Wingender, Flemming, & Mayer, 2004; Haug, 1961; Haug & Smidsrød, 1970; Ouwerx, Velings, Mestdaugh, & Axelos, 1998; Smidsrød, 1974). Gel formation occurs due to an ionic interaction between G residues from 2 or more alginate chains and multivalent cations, typically calcium (Ca^{2+}), to form a three-dimensional network, well described by an "egg-box model" (Grant, Morris, Rees, Smith, & Thom, 1973). Alginates, particularly with high G content, are also extensively used as an immobilisation matrix in encapsulation applications due to their good biocompatibility (Green et al., 2005; Mørch, Donati, Strand, & Skjåk-Bræk, 2006; Skjåk-Bræk & Espevik, 1996; Thu et al., 1996). More recently the potential for alginates in polymer-controlled drug delivery applications has been highlighted (Peppas, 2004; Tønnesen & Karlsen, 2002).

This study is concerned with the investigation of calcium alginate beads, prepared using different conditions of exposure to a calcium chloride gelling bath and the effect that simulated gastro-intestinal (GI) conditions have on the gel microstructure and homogeneity. It is well known that alginate gels prepared by external (dialysis) methods often exhibit a heterogeneous concentration distribution (Smidsrød & Draget, 1996). However, the effect this has on alginate pore size distribution, particularly in simulated GI conditions, is poorly understood. The use of such materials as physiologically responsive hydrogels is dependent on the swelling behaviour of the polymer in GI conditions (Peppas, 2004). We have previously shown that alginate beads shrink in gastric conditions and swell in intestinal conditions due to changes in electrostatic forces brought about by adjusting pH and ionic strength conditions (Rayment et al., 2009). However, in this paper, we utilise spatially resolved NMR (MRI) and TEM to characterise alginate gel networks formed under different conditions to further understand this effect. It has been previously reported that, for dilute polysaccharides

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solutions and gels, the NMR signal arising from the water proton is modulated by exchange between the water protons and protons of macromolecules (polysaccharide chain) (Hills, Cano, & Belton, 1991; Potter, Carpenter, & Hall, 1993). The observed NMR relaxation time, T_2, is a complex function of the number of exchangeable protons on the polysaccharide, the rate of exchange with those sites and the relaxation times of the protons at the exchange sites. NMR transverse relaxation times (T_2) of water protons in polysaccharide gels are considerably decreased compared to bulk water values (Ablett, Lillford, Baghdadi, & Derbyshire, 1976) due to the reduced flexibility of the polymer chains. The state of aggregation and gelation of the polysaccharide determines the extent of this reduction. Therefore T_2 relaxation is a sensitive indicator of the state of gelation and a powerful tool for characterisation of microstructural properties in gels.

This paper describes the effect that both preparation conditions and simulated GI conditions have on alginate gel microstructure and homogeneity using MRI and TEM. Furthermore, we have utilised MR imaging techniques at two different field strengths. This has allowed us to access both high spatial resolution microstructural information at 7 T, as well as probe dynamic microstructural changes at lower field strengths (3 T), which may be utilised *in vivo*. Previous work has highlighted the potential to correlate *in vitro* MRI measurements using a whole-body MRI scanner at 3T with investigation of such systems *in situ* during GI transit (Hoad et al., 2009; Rayment et al., 2009).

2. Materials and methods

2.1. Materials

Sodium alginate (Manugel DMB™) was obtained from ISP Alginates (Tadworth, UK). The average molecular weight of the alginate was determined to be 2.83×10^5 Da using HPSEC-MALLS and the guluronic acid residue content was determined as 72% using ^{1}H NMR analysis. Analytical grade calcium (II) chloride (C3881) was obtained from Sigma–Aldrich (UK) and sodium chloride (S/3160/63) was obtained from Fisher Scientific (UK).

2.2. Preparation of calcium alginate beads

A 1.5% (w/w) sodium alginate solution was prepared by gradually sprinkling the biopolymer powder into deionised water whilst stirring at 22 °C. The solution was stirred for approximately 2 h to ensure complete hydration.

Two types of calcium alginate beads were prepared dependant on the length of time the beads were exposed to calcium chloride. "Strong" (strongly gelled) beads were prepared by dripping 100 ml of 1.5% alginate solution into 400 ml of 0.025 M calcium chloride solution using a 250 ml funnel and 200 μl Gilson tip cut to 39 mm to increase the drip rate. The Gilson tip was held in place using parafilm. Once all the alginate solution had dripped into the calcium chloride solution, the beads were sieved and rinsed in fresh calcium chloride solution after 5 min. The beads were placed into a storage container with 400 ml fresh calcium chloride solution (control solution) and stored at 5 °C for 22 h before characterisation. Weak (weakly gelled) beads were prepared by dripping 100 ml of 1.5% alginate solution into 400 ml of 0.025 M calcium chloride solution for 2 min. The alginate beads remained in the calcium chloride solution for a further 3 min with gentle stirring before being sieved and rinsed with 0.05 M sodium chloride solution. Finally the beads were stored in 400 ml fresh sodium chloride solution (control solution) at 5 °C for 22 h before characterisation. Following storage, the bead diameter was determined as described in Rayment et al. (2009). The strong beads were smaller (approximately 3.9 mm) compared to the weak beads (approximately 4.3 mm) reflecting an increased syneresis of the gel at high calcium concentrations (Kim, 1990; Martinsen, Skjåk-Bræk, & Smidsrød, 1989).

2.3. Protocol for simulating gastric and intestinal conditions

Solutions were prepared to be physiologically similar to gastric and intestinal juices for a fasted human (Altman, 1961). The beads were exposed to simulated gastric conditions (2.86 g/L NaCl, 0.865 g/L KCl and 0.4 g/L $CaCl_2$; all Sigma–Aldrich Analytical Grade Chemicals) for 2 h at 37 °C. The pH of the gastric solution was reduced to pH 2.0 using 4 M HCl (Fluka 84435) after 15 min to simulate *in vivo* acidification. The beads were then exposed to simulated intestinal conditions (6.5 g/L NaCl, 0.835 g/L KCl, 0.22 g/L $CaCl_2$ and 1.386 g/L $NaHCO_3$) for 3 h. These experiments were conducted using a simulated intestinal solution containing simple salts and a pH close to that found in parts of the human intestine. Bile extract was not included in these studies and we expect the gel integrity to be further weakened by their inclusion. This is investigated in future studies. The final pH of the intestinal phase solution was approximately 8.0. Both solutions were made 24 h before an experiment.

2.4. ^{1}H MRI at 7 T for high resolution imaging of beads

^{1}H 2D NMR images were recorded on a Bruker DSX 300 spectrometer (operating at a proton frequency of 300 MHz) with a Bruker Avance console and equipped with a standard micro-imaging Micro 2.5 probehead and a 15 mm RF birdcage resonator.

One or more alginate gel beads of the same type were analysed simultaneously while immersed in the appropriate solution. In a typical experiment, the alginate beads were placed in an NMR tube horizontal slice images through the centre of the beads were obtained using a standard single spin–echo imaging sequence. The slice thickness was 1 mm; field of view was 15×15 mm^2; and in-plane voxel resolution was 59×59 μm^2. The recycle time was 10 s and the echo time (*TE*) was typically 40 ms with total scan time lasting approximately 42 min.

For spatially resolved measurements of the transverse relaxation time T_2 of the water protons within the gel, separate images were acquired at 24 echo times (*TE* = 10–240 ms in steps of 10 ms). All other imaging sequence parameters remained constant between images. The maps of initial magnetisation M_0 and of relaxation time T_2 were obtained by fitting a single exponential function to the signal decay M of each pixel, $M = M_0 \exp(-TE/T_2)$ + constant. Radial averages of M_0 and T_2 for each bead were extracted by averaging the corresponding maps in 'shells' from the centre of the bead towards the surface.

Strong and weak alginate beads were exposed to solutions simulating gastric and intestinal conditions as described previously. An individual bead was followed throughout the experiment, separated from the others using a small sieve. It was scanned at the end of each phase of exposure (i.e. control, gastric and intestinal conditions), when it was removed from the main sample and immersed in a small quantity of appropriate solution.

Profiles across the images of the beads were made by radial averaging, so that each point on the profile was obtained by averaging the signal in the map in 'shells' from the centre of the strong bead towards the surface.

2.5. Magnetic resonance imaging (MRI) at 3 T

Data were acquired on a 3.0 T Philips Achieva (Philips Medical Systems) whole body scanner with an eight element SENSE-Head coil. A T_2-weighted high resolution Turbo Spin Echo (TSE) sequence