Macroporous alginate gels

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ABSTRACT

Alginate is a biocompatible, renewable polymer extracted from seaweed. The readily available anionic polysaccharide find commercial use in diverse areas such as wound care, pharmaceutics and food. Alginate forms a gel by rapid crosslinking with di- and tri-valent ions [1]. In this work we have used two different ways to introduce the multivalent ions that act as ion bridges between alginate. The methods are the "internal setting" [1] of the gels giving macroscopically homogeneous gels and “directed external” gelation [2,3] of the alginate giving rise to anisotropic growth of open capillaries running through the gel. While the rheological properties of the internally set alginate gels have been studied extensively [4], those of the directed externally set gels, exhibiting open capillaries, have been studied to less extent [5]. Here we compare the stress relaxation of directed external alginate gels and internally set alginate gels. We show that the externally set alginate gels are plastic in behaviour. Further, we show that this plasticity is not correlated to the presence of open capillaries *per se* but rather related to the network structure.

INTRODUCTION

Alginate is a readily available biopolymer used as a biomedical material (e.g. in tissue engineering [2], cell immobilisation [6]) and has additional large field of application as thickener and gelling agent in diverse fields such as food, pharmaceutics and waste water treatment [4]. Alginate is biocompatible, of relatively low cost and form gels at mild conditions.

Alginate gels are typically formed by so called internal gelation, where an insoluble (calcium) salt is used in combination with a slowly released acid such as glucono delta lactone. A slow introduction of the crosslinking ion (in this case calcium) yields macroscopically homogeneous gels. Macroscopically inhomogeneous gels containing parallel aligned capillaries running from top to bottom of the gel can be obtained by anchoring of alginate to a surface, after which crosslinking ions (e.g. calcium) are allowed to diffuse into the alginatge solution via a gelled membrane (directed external gelation [3,5]) (Figure 1).

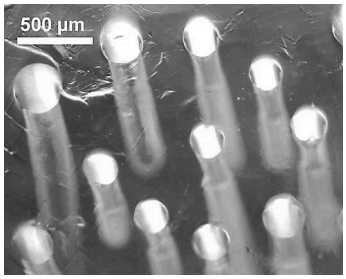

Figure 1: Directed externally set calcium alginate gel with capillaries in the size range of 200 to 250µm.

The diameter of the capillaries can be controlled (from 20 to 400 µm) by the choice of crosslinking ion , concentration of crosslinking ion and alginate concentration [5].

In this work, we report on the mechanical properties of macroscopically inhomogeneous alginate gels formed using the above method.

MATERIAL AND METHODS

Alginate solutions (2 % w/w) were prepared by careful addition of alginate powder to deionized water at room temperature under vigorous stirring. The dispersion was thereafter heated to 353 K in a water bath and kept at this temperature for 30 minutes or until dissolution was obtained. The pH of the solution was adjusted from pH 7.3 to pH 7 using 0.1 M HCl.

Gel preparation: Alginate gels using internal setting were prepared by controlled release of calcium. $CaCO_3$ and GDL were rapidly dispersed in water and immediately added to the alginate solution to a yield final alginate concentration of 1.5 %. The dispersions was poured into cylindrical Teflon moulds (h = 12.5 mm; d = 12.5 mm). The moulds were sealed and the samples were allowed to equilibrate and set at room temperature for 48 h prior to use. It is important to note that the GDL was always used in stoichiometric equivalence to $CaCO_3$ (e.g. 15 mM $CaCO_3$ and 30 mM GDL) to keep the pH constant during network formation. The calcium alginate networks formed via internal setting was R = 1 where R is defined according to

$$R = 2[Ca^{2+}]/[guluronate]$$

Externally set alginate gels were prepared by coating the internal wall of a glass beaker (V = 50 ml and d = 40 mm) with alginate by brushing the internal wall with alginate solution, which then was allowed to dry in an oven set at 383 K for 30 minutes. The proceeding was repeated three times. A volume of 20 ml alginate solution (1.5 % w/w) at 293 K was poured into the glass beaker. The surface of the solution was sprayed with $CaCl_2$ solution until a gel membrane was formed on top of the alginate solution. The gel membrane was left to set for 30 minutes after which $CaCl_2$ solution at 293 K was carefully poured on top of the membrane. The $CaCl_2$ solution was left to diffuse through the membrane and into the alginate solution for 48 h prior use of the gel.

Large deformation rheology: Uniaxial compression tests were performed on all gels using an Instron mechanical test frame (model 5565A). At least three repeats were done for each sample. The gel cylinders tested were in the case of the internally set alginate carefully removed from the mould and aligned in the centre of stainless steel compression plates, which were lubricated with mineral oil to reduce friction. In case for the externally set gel, the gel was carefully removed from the beaker, after which the membrane was removed using a razor blade and cylinders were stamped out from the top part of the gel (after removal of the membrane). Each gel was carefully examined for cracks or deformation obtained from handling prior testing. The gels were aligned in the center or stainless steel compression plates as for the internally set alginate gels. Stress relaxation test were performed by compressing the samples to 10% strain using an initial crosshead speed of 4 % strain / second. The stress response upon relaxation of the gel was studied for up to 15 minutes.

RESULTS AND DISCUSSION

We have previously shown that externally set gels with capillaries are plastic in behaviour compared to internally set alginate gels at similar calcium concentration. Here we prepared externally set gels where the concentration of the ion solution diffusing into the alginate is too low to yield capillaries, yet high enough to form a gel. Our aim was to elucidate whether the network structure on its own is more plastic in behaviour compared internally set alginate gels.

Stress relaxation of the internally and externally set gels (absence of capillaries) are shown to behave differently (Figure 2). The externally set

gels are more plastic in behaviour to the internally set gels. Furthermore, the network appear anisotropic as gels cut along the inflow of ions relaxes in a different way to those cut perpendicular to the inflow of ions.

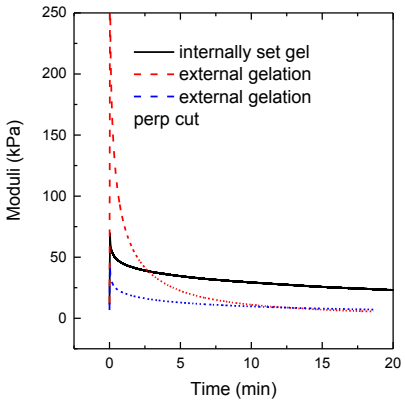

Figure 2: Stress relaxation of internally and externally set alginate gels at a strain of 10%.

CONCLUSIONS

Stress relaxation tests on externally set alginate gels in the absence of capillaries show a plastic behaviour of the gel. Furthermore, the gel network appear anisotropic judging from the different stress relaxation behaviour obtained between differently cut gels.

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