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Interferometric characterization of swelling of covalently crosslinked alginate gel and changes associated with polymer impregnation

Sven Tierney [a], Marit Sletmoen [a], Gudmund Skjåk-Bræk [b], Bjørn Torger Stokke [a,*]

[a] Biophysics and Medical Technology, Department of Physics, The Norwegian University of Science and Technology, NTNU, NO-7491 Trondheim, Norway
[b] Department of Biotechnology, The Norwegian University of Science and Technology, NTNU, NO-7491 Trondheim, Norway

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ABSTRACT

We report on integration of covalently crosslinked alginate hydrogels at the end of an optical fiber suitable for determination of changes in optical length with resolution of 2 nm, and the characterization of such alginate hydrogels, in particular following impregnation with a polycation. The ionic strength dependence of the changes in the optical length reflect the swelling, and the results showed typical expansion of swelling volume on reducing the ionic strength for an ionic hydrogel. Polycation impregnation of the alginate hydrogels by immersion in aqueous solution containing either poly-L-lysine or chitosan resulted in changes of the optical signal during the impregnation differing between the two polycations. The ionic strength dependence of the hydrogel swelling was also affected. A strategy for modelling of the resulting swelling of the impregnated hydrogel, aiming at a method for nanomechanical characterization of the supported impregnation is suggested.

1. Introduction

Application of an interferometric based readout platform has proved to provide high resolution data of changes of the optical pathlength within a hydrogel (Tierney, Hjelme, & Stokke, 2008; Tierney, Volden, & Stokke, 2009). In a typical geometrical arrangement, the gel is immobilized on a glass surface, and incident, nearly monochromatic light through the glass is partially reflected and partially transmitted at the interfaces. The reflected waves from the glass–gel and gel–solvent interfaces towards the direction of the incident light, yields a net interference wave with a phase that depends on the difference in the optical pathlength of the two reflected waves thus providing experimental access to the optical pathlength within the gel. Realized at the end of an optical fiber, and using a wavelength packet 1530–1560 nm, we have previously reported determination of changes in the optical length of a hydrogel with a resolution of 2 nm (Tierney et al., 2008). In view of the typical length of about 50 μm of the hemispherical gels synthesized at the end of the optical fiber, the technique supports relative length changes of the hydrogel with resolution of the order 0.005%.

We have previously reported on the application of this interferometric technique for the high resolution determination of the ionic strength, *I*, dependence and pH dependence of both the equilibrium swelling of a cationic gel (Tierney et al., 2008). The technique with a sample rate of about 1 Hz was also suitable for the determination of the kinetics of the gel networks swelling following stepwise changes in either ionic strength or pH. Further work has focused on the integration of recognition elements supporting changes in functionalized hydrogel swelling in the presence of various sugars (Tierney, Falch, Hjelme, & Stokke, 2009; Tierney, Volden, & Stokke, 2009) or binding to oligonucleotide sequences (Tierney & Stokke, 2009). Both the glucose sensitive gels and the hydrogels recognizing oligonucleotide sequence mediated changes in the swelling response through the binding effect of the probe to affect the cross-linking density. For the degrees of swelling observed, the transformation of Δl_{opt} to changes in physical dimensions in these cases appeared to be well approximated by dividing by a constant equal to the average of the refractive index of the gel for the range of swelling observed. In the present work we explore the possibility to use the high resolution interferometric technique to study the swelling properties of polymer gel materials applied for immobilization of living cells. In particular, the ability to study changes associated with exposure of these hydrogels to polymers used in the immobilization procedure to enhance the properties of the gel based immobilization are investigated.

Immobilization of living cells employing ion-induced gelation of alginates are established as an important part of preparing immunoisolated cells necessary for exploration for transplantation (Morch, Donati, Strand, & Skjak-Braek, 2007; Smidsrød & Skjåk-Bræk, 1990). In particular, since Lim and Sun first described a bioartificial pancreas of pancreatic islets in alginate-poly-L-lysine capsules in 1980 (Lim & Sun, 1980), alginate-polycation capsules have been the most studied system for microencapsulation of insulin

* Corresponding author.
E-mail address: bjorn.stokke@ntnu.no (B.T. Stokke).

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producing cells. The successful use of alginate-polycation capsules as carriers for insulin producing cells *in vivo* imply strict requirements on the capsules' biocompatibility as well as their mechanical stability. Major challenges have been their sensitivity to calcium sequestrants and non-gelling ions such as sodium, the osmotic swelling under physiological conditions, the high porosity and wide pore size distribution and exposed polycations on the surface (Strand, Mørch, Syvertsen, Espevik, & Skjåk-Bræk, 2003b; Strand et al., 2001). The use of a polycationic impregnation of the initial alginate gel bead formed poses challenges on the biocompatibility, and development routes abolishing the polycationic impregnation are in focus (Morch et al., 2007). Nevertheless, the claimed mechanical stabilization and possibility to modulate the porosity as a part of a barrier function are positive features that one can take advantage of. While various tools for the study of the biocompatibility of cell encapsulation materials and microcapsules have been developed and applied, the determination of the mechanical properties of the polycationic layer deposited on the surface appears more difficult. Mechanical testing of polyelectrolyte multilayers and reinforced polymer gels are challenging in the sense that they constitute soft materials of very small dimensions, where nanomechanical characterization appear to be the most viable route. In the following, the potential of the high resolution interferometric technique to support high resolution determination of ionic strength dependence of the gel material before and after the polycationic exposure will be explored. The long term aim is to establish the technique as a tool that, in combination with other techniques and proper analysis, can be applied for the determination of the mechanical properties of the composite material deposited upon polycationic impregnation of the hydrogel materials.

2. Materials and methods

2.1. Materials

Alginate gels were prepared at and covalently linked to the end of an optical fiber using a methacrylate-grafted alginate prepared as reported (Rokstad et al., 2006). Briefly, the high molecular weight mannuronan (fraction of guluronic acid residues less than 0.1%) isolated from an epimerase negative mutant of *Pseudomonas fluorescens*, was modified by dropwise addition of methacrylic anhydride to a 1% mannuronan aqueous solution (pH 9, $T = 0$ °C). The solution was stirred (8 °C, 24 h) and aqueous NaCl was added to 1%. The resulting modified polymer was precipitated, centrifuged and washed using ethanol. Salt was removed by dialysis. The freeze dried methacrylate mannuronan was dissolved and epimerised using mannuronan C5-epimerase AlgE6 (Holtan, Bruheim, & Skjak-Braek, 2006) to introduce guluronic acid. The epimerisation was carried out at 0.25% alginate in 50 mM MOPS, pH 6.9, 2.5 mM Ca^{2+} and 75 mM NaCl buffer at an AlgE6 to alginate weight ratio 1:100, for 25 h at 37 °C (Holtan et al., 2006). The characterization of the sample by ^{1}H NMR (Grasdalen, 1983) showed a fraction 0.28 guluronic acid residue was obtained, and a 5% degree of methacrylate substitution was obtained. The employed procedure yields methacrylate only on the mannuronic acid of the final sample.

A chitosan with a degree of acetylation $F_A = 0.1$, and estimated molecular mass $M_w = 33 \times 10^3$ g/mol based on characterization of solution properties was employed. This sample was kindly provided by Dr. K.M. Vårum, Dept. of Biotechnology, NTNU. The chitosan was dissolved in acetic acid (1%), to a concentration of 1 mg/ml. A poly-L-lysine (PLL) $M_w = 22 \times 10^3$ g/mol has been found to be optimal based on capsule stability and permeability (Thu et al., 1996) and this M_w of PLL was therefore used to prepare multilayers. The PLL (Sigma Aldrich) was dissolved in MQ water.

Additional chemicals employed for surface functionalization of the optical fibers and gelation were hydroxycyclohexylphenylketone (99%, Aldrich), squalane (99%, Aldrich), and plusone repel-silane (Amersham biosciences). Water (resistivity 18.2 MΩ cm obtained using a Millipore water purification system) was used for all solutions, sodium chloride (Sds, 99%), sodium hydroxide (99%, Merck), hydrochloric acid (37%, Merck) and MES hydrate (99.5%, Sigma).

2.2. Preparation of the alginate gels covalently attached to the optical fiber

The optical fibers were stripped of the jacket, cleaned with 96% ethanol and then immersed in a solution of plusone repel-silane for 20 min to hydrophobically modify the fiber surface. The fiber was cut (Fitel Model s323, Furukawa Electric Co. Ltd.) and prepared for silanization of the fresh surface (Cras, Rowe-Taitt, Nivens, & Ligler, 1999) by immersing the fiber in aqueous 1.0 M NaOH (Merck) for 20 min to remove impurities on the surface, followed by rinsing in water. The fiber surface was subsequently activated for silanization (immersed in aqueous 0.01 M HCl (diluted from 37%, Merck)), and finally soaked in a solution of 3-(trimethoxysilyl) propyl methacrylate (Sigma, >98%) (0.02 M, nitrogen purged water pH 3.5) for 1 h, chemically binding the methacrylate groups to the fiber tip. The methacrylated alginate was dissolved in MQ water at 4.5 wt.% and an aliquot of this solution was deposited at the end of the functionalized optical fiber immersed in squalane with the photo-initiator. The light guide connected to a UV source (Dymax Bluewave 50 light curing system), was pointed towards the alginate pregel solution within the squalane droplet. The methacrylated alginate pregel solution was cured by exposing it to the UV light for 11 min. The resulting hemispherical alginate gel was briefly immersed in a hexane solution to remove the oil, and then washed in the buffer solution for at least one day to eliminate impurities.

The alginate gels at the fibers were immersed in an aqueous solution where solvent conditions (ionic strength, polycationic solution for gel impregnation) were changed. The determination of gel swelling as a function of ionic strength were carried out using stock solutions of various concentrations as reported previously (Tierney et al., 2008). The covalently attached alginate gel on the fiber was also subject to polycationic impregnation by immersing the alginate gels in aqueous solutions of the polycations (poly-L-lysine, chitosan) dissolved in the given aqueous solutions, diluted and adjusted to the solution conditions used for the deposition conditions.

The hydrogel materials deposited at the end of the optical fiber were characterized by an optical interferometric technique. The incident light from a source bandwidth of 1530–1560 nm with intensity I_0 reflected both from fiber–gel (reflectivity r_1) and gel–solution interface (reflectivity r_2), yields a net interference wave with intensity:

$$I(\lambda) = I_0(\lambda)\left[r_1^2 + r_2^2\gamma + 2r_1r_2\gamma^{1/2}\cos\left(\frac{4\pi l_{opt}}{\lambda} + \phi\right)\right] \quad (1)$$

In Eq. (1), γ is a loss factor of the gel-cavity, l_{opt} is the optical length of the gel, λ is the wavelength and ϕ is an initial arbitrary phase. Hydrogel swelling yields changes in the optical path length l_{opt} of the gel thus inducing changes both in the intensity and the phase. The change in the optical length Δl_{opt} is determined from the phase change of the interference signal:

$$\Delta l_{opt} = \Delta\phi\lambda_0/4\pi \quad (2)$$

$\Delta\phi$ is the phase change, λ_0 is the center wavelength of the source spectrum. The net changes in Δl_{opt} through the gel arises from differences of the integral of the refractive index of the gel n_{gel}

along the lightpath, i.e., the technique provides the net change, and does not provide information about eventual inhomogeneous changes of n_{gel}. In the situation of homogeneous materials, the net homogeneous changes can be viewed as differences in n_{gel} and the physical length, l (Tierney et al., 2008).

3. Results and discussion

Fig. 1 shows the change in the optical length of the covalent crosslinked mannuronan hydrogel attached to the end of the optical fiber versus the ionic strength in solution. The l_{opt} of this hydrogel was originally 86 μm at high ionic strength and increased by reducing the ionic strength to an apparent plateau value below 2 mM ionic strength, with a value of l_{opt} = 120 μm. This corresponds to a relative change of l_{opt} of 30%. The corresponding change in l_{opt} for synthetic cationic gels are about 7.5% and 5.8% for the hydrogels reported on previously (Tierney et al., 2008). This indicates that the optical technique provides experimental data that displays changes in gel swelling following the trend as expected for polyelectrolyte gels. The more strongly charged gels respond with larger changes in the swelling volume than the less charged ones. Although not explicitly explored here, changes in the crosslinking density of the gels are also expected to affect the observed changes in l_{opt}. The magnitude of the change in l_{opt} for the alginate determined here is considered to be consistent with the relative swelling volume change of −(50–60)% from $I = 10^{-4}$ M NaCl to I = 1 M reported for other covalent crosslinked alginates (Moe, Skjåk-Bræk, Elgsaeter, & Smidsrød, 1993). Such data can be the basis for more quantitative analysis of the swelling response where network charge density parameters, crosslink density, finite extensibility of chains and overall mass concentration are used as model parameters (Guo, Elgsaeter, Christensen, & Stokke, 1998). This would require the proper conversion of l_{opt} to swelling ratio, including e.g., potential effects on the refractive index at different l_{opt} (Tierney et al., 2008). Continuous monitoring of l_{opt} following a step change in ionic strength of these alginate gels was observed to have time constants in the order of a few seconds, which is similar to that reported previously for synthetic gels (Tierney et al., 2008). The present work explicitly demonstrates that a certain type of alginate can be used as the base polymer gel material on the fiber-optic readout platform, thus supporting a range of alternative characterizations of alginate, and possibly other biopolymers, by the interferometric platform.

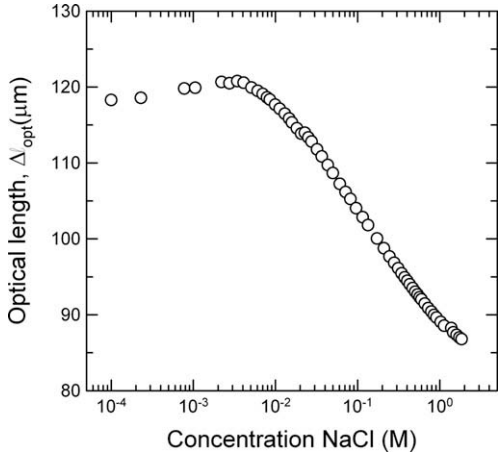

Fig. 1. Optical length of the hemispherical, covalently crosslinked, 4.5 wt.% mannuronan hydrogel immobilized at the end of the optical fiber versus the concentration of NaCl in the immersing aqueous solution.

The covalently crosslinked alginate hydrogels were subject to polycationic impregnation by a poly-L-lysine or a chitosan. These polycations were selected since they are explored for modulating the barrier functionality within alginate gel used for immobilization of living cells (Strand et al., 2001; Thu et al., 1996). In Fig. 2, the amplitude of the optical signal and the change in l_{opt} is shown versus time during the deposition of these two polycations from an aqueous solution. The data for the optical amplitude signal shows an increase with time for both the deposited polycations, with the largest change occurring within the first 5 and 10 min for the chitosan and poly-L-lysine, respectively, followed by a plateau of larger amplitude. Exposure to the chitosan solution, however, induced hardly any change in l_{opt}, whereas the poly-L-lysine solution induced a constantly decreasing l_{opt} with time during the deposition. Both of these trends can be explained by Eq. (1). The increase in amplitude is most likely due to the increase in r_2 due to a larger polymer concentration on the gel surface. The impregnation employing chitosan can be interpreted to yield a large r_2 possibly by a larger overall polymer concentration gradient between the outermost layer and the surrounding solution than the impregnation employing poly-L-lysine. On the other hand, l_{opt} will be affected by both changes in the physical length of the gel-cavity and the mean refracted index of the gel. This may arise due to an elastic contribution imposed by the deposited layer only seen for the poly-L-lysine in this project. However, a more detailed interpretation awaits further experimental evidence of the actual polymer concentration profiles using e.g., fluorescent samples following the impregnation.

The experiments displayed in Fig. 2 are selected from several series where the additional following trends were deduced. Increasing the concentration of the polycation induces so rapid changes in the hydrogel based material that the interferometric readout technique is not suitable for the continuous monitoring of the induced changes.

The alginate hydrogel cores with the polycationic impregnation were subsequently characterized by the ionic strength dependence of l_{opt}. The data show that l_{opt} for the poly-L-lysine coated alginate hydrogel (Fig. 3) depends more strongly on the ionic strength than the non-treated hydrogel. The chitosan impregnation procedure did not appear to induce a significant change in such type of data (not shown), although a significant change was observed for the amplitude of the optical signal (Fig. 2). In a further work we aim

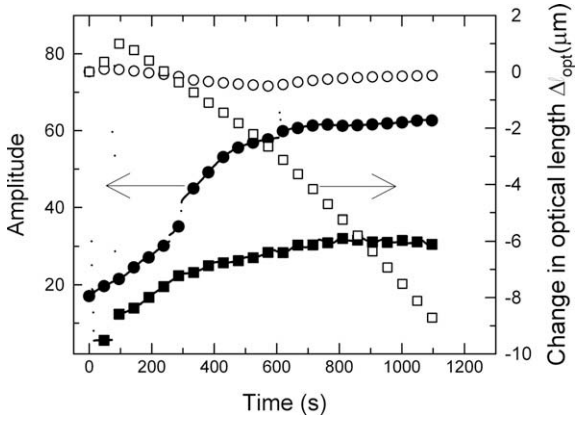

Fig. 2. Amplitude of optical signal (filled symbols, left axis) and change of optical length (open symbols, right axis) versus time for impregnation of a covalently crosslinked alginate hydrogel attached to an optical fiber with chitosan (●, ○) and poly-L-lysine (■, □). Large symbols are shown for every 50th datapoint collected, and the apparent lines between the datapoints for the amplitude depicts all collected data. Polycation deposition was carried out employing 35 μg/ml polycation, pH 5.5 in MES buffer.

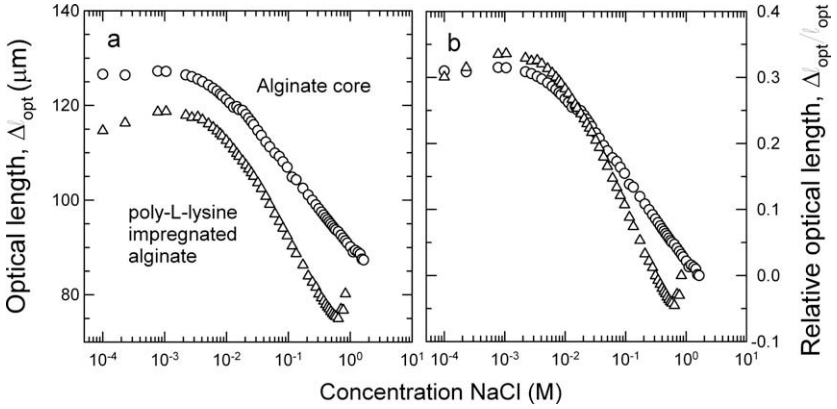

Fig. 3. Optical length (a) and relative optical length (b) of covalently crosslinked alginate hydrogel attached to an optical fiber and following poly-L-lysine impregnation (35 mg/ml poly-L-lysine, pH 8). The normalisation in Fig. 3b is for both gels with respect to the optical length in 1 M NaCl.

at addressing the effect of the polycation impregnation of the hydrogel material to determine the mechanical effect of the polycation impregnation. This will be based on the theoretical description of hydrogel swelling (Shibayama & Tanaka, 1993; Treloar, 1975), which we also have applied previously (Guo et al., 1998), extended to include effects of the polycationic impregnation. In a first approximation, the observed effect of the coating can be included as a shell effect where the polycationic impregnation affects the swelling pressure of the gel (Ottøy, 1996). This approach is similar to the core-shell approach used to describe a mechanical skin effect associated with selective adsorption of cationic surfactant in the outermost layer of a polyanionic hydrogel (Hansson, Schneider, & Lindman, 2002).

The responsive character of the hydrogel core employed here can be conceptually outlined from the theory of swelling of hydrogels. Briefly, in a first approximation, the equilibrium of a polyelectrolyte hydrogel is described by total zero osmotic pressure (Π) of the gel. The osmotic pressure of an ionic hydrogel is conventionally described by three additive contributions arising from the free energy of mixing of the polymer with the solvent, Π_{mix}, secondly, the elastic retractive force associated with deformation of the polymer chains, Π_{el} and thirdly, the difference in mobile ions concentration inside and outside of the gel, Π_{ion} (Shibayama & Tanaka, 1993; Treloar, 1975):

$$\Pi_{mix} + \Pi_{el} + \Pi_{ion} = \frac{RT}{V_1}(\ln\varphi_1 + \varphi_2 + \chi\varphi_2^2) + \frac{\upsilon RT}{V_0}\left(\frac{\varphi_2}{2\varphi_{2,0}} - \left(\frac{\varphi_2}{\varphi_{2,0}}\right)^{1/3}\right) + RT\Delta C_{tot} \quad (3)$$

In Eq. (3), subscripts 1 and 2 of the volume fractions φ denote the solvent and polymer phase, V_1 is the molar volume of the solvent, υ is the molar number of elastic active polymer chains in the gel at the reference volume fraction $\varphi_{2,0}$, V_0 is the gel volume for the reference state, R is the molar gas constant, T is the absolute temperature, and χ is the Flory–Huggins interaction parameter. The total difference in molar concentration of mobile ions between the gel and the surrounding aqueous solution, ΔC_{tot}, is given by the Donnan equilibrium. In a first approximation, the impregnation can be approximated as a thin spherical layer with tension of the shell. This tension can be represented as an additive contribution to the overall equilibrium:

$$\Pi_{mix} + \Pi_{el} + \Pi_{ion} + \Pi_{shell} = 0 \quad (4)$$

Parameter Π_{shell} can subsequently be modelled applying analogous material parameters as for the gel core, but with the appropriate parameters values valid for the surface layer. The combined quantitative fit of such an approach to the hydrogel core swelling, similar as we have reported previously (Guo et al., 1998), and similar measurements for the impregnated gel material, may be a viable route for the determination of material properties of the impregnation layer supported by the gel core. Hansson and coworkers outlined a similar strategy to account for a shell effect of polyanionic (polyacrylic acid) hydrogels exposed to polycationic surfactant. They implemented a rubber-like elasticity for the shell effect, and used parameter values for the polyacrylic core hydrogel to model the effect of the shell in the coupled core-shell model (Hansson et al., 2002). They reported a larger quantitative effect of the shell than in our results. The present results of alginate gel deswelling following exposure to poly-L-lysine is also similar to polyacrylic acid hydrogel deswelling on exposure to poly-L-lysine (Bysell, Hansson, & Malmsten, 2008), although the magnitude of the deswelling is less in the present data.

The outlined approach should preferentially be complemented by additional experiments addressing the relative polymer distribution in the composite materials. This need is indicated by the reported layer thickness of poly-L-lysine and chitosan coated alginate gel used for immobilization of living cells (Chen, Wei, Bisi, Martoni, & Prakash, 2005; Strand, Mørch, Espevik, & Skjåk-Bræk, 2003a). Such data are planned to be integrated in the modelling. The data reported here indicate analogous effects of polycationic impregnation of alginate gels as previously observed for polymer gel coating of poly-*N*-isopropylacrylamide gels (Greinert & Richtering, 2004; Wong & Richtering, 2008) in affecting the swelling behaviour. The outlined strategy may therefore complement existing approaches (Richert, Engler, Discher, & Picart, 2004; Vinogradova, Andrienko, Lulevich, Nordschild, & Sukhorukov, 2004) for nanomechanical characterization of supported thin polyelectrolyte films, with interest also for e.g., hydrogels displaying phase changes.

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