A novel device was designed to perform electrophoretic deposition under tightly controlled conditions. The device parameters were investigated by depositing three different viscosity grades of sodium alginate hydrogels. A thin metallic rectangular substrate was used to obtain the various time dependent deposition rates of the gels. The resulting deposition curves showed the effective electrophoretic mobilities of the low, medium and high viscosity grade gels to be 0.0610 cm²/Vs, 0.0584 cm²/Vs and 0.0909 cm²/Vs, respectively. The ratios of gel deposit to solution resistivities were 21.0, 16.2 and 47.5 respectively. Following electrophoretic deposition, the gels were cross-linked in a 0.1 M CaCl₂ solution in order to further solidify the gels. Cross-linking reduced the masses of the gels to 50.9 ± 1.8%, 26.7 ± 2.0%, and 28.5 ± 1.3% of their initial masses respectively. Lysophospholipidization was applied to the gels to determine the alginate content of the gels. Immediately after deposition the alginate mass fractions were 5.59 ± 0.07%, 7.11 ± 0.37% and 7.02 ± 0.24% respectively. The device in this study provided sufficient data to model the electrophoretic deposition rates. The technique can be expanded to other hydrogel species which can be used in a variety of biomedical and biotechnological applications.

Keywords: Alginate; Electrophoretic deposition; Modelling; Hydrogel; Tissue Engineering; Encapsulation; Rectangular Substrate

Introduction

Electrophoretic deposition (EPD) is a relatively inexpensive method of forming uniform layers or bi-layers on substrates with complex geometries. It has gained much attention in the last 20 years in the development of advanced materials including functional and structural ceramic coatings [1], laminated ceramics [1], biomaterials [2,3], composites [3-6], porous materials, thin films, and nano-structured materials [6].

In this method, particles are suspended or ions are dissolved in solution before they are driven towards a substrate through the application of an electric field. The particles/ions then form a solid film on one of the electrodes through a number of possible mechanisms including flocculation through accumulation [7], particle charge neutralization [8], particle coagulation [6], and through electrical double layer thinning [9]. EPD has been used on a number of biomaterials such as bio- ceramics, bioactive materials, nanoparticles, and hydrogels [2]. Porous materials, composites, and textured layers have all been produced for biomedical engineering purposes using this technique.

Sodium alginate is a polysaccharide copolymer made of (1-4)-linked β-D mannuronate and C-5 epimer α-L-guluronate blocks. To date, our laboratory has focused on the therapeutic applications of encapsulated alginate systems. The hydrogels derived from brown algae and has many uses in a variety of fields including tissue engineering. Alginates can be used for both in-situ gelation and immobilization-through-microencapsulation to create injectable scaffolds [10]. Such methods have already been used for bone tissue repair [11], cartilage repair [12], skin repair [13] and in neural tissue repair [14]. Recently, alginate has been used to prevent adverse tissue remodeling in damaged myocardial tissue [15]. Alginates are also used for drug delivery [16], cell delivery, enzyme encapsulation and wound dressing [17]. This class of materials is widely used for its non-toxicity, biodegradability, and excellent biocompatibility [16].

There are several alginate viscosity grades available which, along with the G/M block ratio, the concentration of cations and the cation species employed during the cross-linking process, greatly affect the gel mechanical and swelling properties [16,18]. The weight average molecular weight has been correlated with viscosity [19] which was shown to have a large impact on the drug release rate of the gel under neutral pH [20,21]. The erosion of the gel has been shown to vary depending on the acidity of the environment [22,23]. Previous research has also focused on the importance of cross linking with either Ca²⁺ or Ba²⁺ ions in order to improve the mechanical stability of alginate gels [24].

The negatively charged alginate particles are amenable to EPD which results in the formation of a thin uniform alginate-gel film on the desired substrate [7,13,25,26]. Previous studies have focused on the EPD of sodium alginate for biosensor, tissue engineering and corrosion resistance applications [17,26-28]. Sodium alginate undergoes an anodic deposition, and the mechanism has been previously described in literature [25].

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Although the feasibility of alginate deposition and co-deposition has been well established, more research is needed to better understand the characteristics of the deposition itself. To the best knowledge of the authors, no study has yet been published that investigated the electrophoretic mobility and the resistivity ratio between the deposited gel and the solution, both of which are required to model the deposition rates. This would allow for a more precise control over the gel thickness which is important for industrial applications. Moreover, the impact of alginate viscosity on EDP has not yet been addressed even though it greatly affects properties such as drug release rate, swelling, and mechanical strength of the gel when it is prepared through direct cross-linking alone.

Materials and Methods

Materials

Three viscosity grades of alginates were used for deposition: low viscosity (LV) alginic acid sodium salt (MP Biomedicals, Solon, Ohio, USA) which was rated at 250 cP at 2% aqueous, medium viscosity (MV) alginic acid sodium salt (Sigma Aldrich, St. Louis, Montana, USA) which was rated at 3,500 cP at 2% aqueous, and a high viscosity (HV) alginic acid sodium salt (Sigma Aldrich, St. Louis, Montana, USA) which was rated at 14,000 cP at 2% aqueous. The alginates were chosen in order to span the viscosity grades that are commonly manufactured. All alginates were derived from the kelp species M. pyrifera.

Method for determining alginate deposition rates

The EPD device was used to investigate the deposition rates of the three different viscosity grades of alginate. After alginate was deposited on the substrate, the film was rinsed in deionized water before it was briefly exposed to compressed lab air to remove droplets from the surface. The mass was measured before and after deposition using a Mettler Toledo AG204 Delta Range scale (Mettler-Toledo, Greifensee, Switzerland). The mass of the substrates and films were recorded at 1, 2, 3, 5 and 10 minutes. Approximately 1.5 L of solution was used to ensure the alginate concentration remained approximately constant. Depositions were carried out in triplicate at room temperature under static conditions.

Method for cross-linking and evaluation of mass

Cross-linked gels were obtained by submerging the post-deposit gels in a 0.1 M CaCl₂ (ACP, Montreal, Quebec, Canada) solution overnight at 4°C. The gel masses were evaluated before and after cross-linking and samples were obtained in sextuplicate while the gels were still attached to the substrate.

Method for lyophilization and dry gel mass through

After deposition, sample gels were removed from the substrate, weighed, and placed in petri dishes. A Thermo Savant ModulyoD-115 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) was used to freeze-dry the samples over the span of two days after which changes in mass were recorded. Samples were obtained in triplicate.

Method for determining molecular weight through gel permeation chromatography

Gel permeation chromatography (GPC) was used to differentiate the alginate powders by molecular weight. Experiments were performed courtesy of the Laboratoire de Caractérisation des Matériaux Polyméres du Département de chimie at L'Université de Montal. The weight average molecular weight (Mₙ) the number average molecular weight (Mₘ) and the size average molecular weight (Mₚ) of the three alginate powders were obtained. Samples were dissolved in a 0.1 M NaNO₃ solution at a concentration of 10 mg/ml, and experiments were carried out at 35°C. A Polysep-5000 GPC setup (Phenomenex, Torrance, California, USA) with an Ultra hydrogel 500 (Waters, Milford, Massachusetts, USA) column was used with Breeze v3.20 software (Waters, Milford, Massachusetts, USA). The molecular weights were subsequently used to calculate the viscosities of the different alginate grades using the Mark-Houwink-Sakurada equation.

Method for determining contact angles of dried alginate gels

Contact angles were obtained using a VCA Optima (Billerica, Massachusetts, USA) goniometer with 0.25 μL water droplets. Gels were allowed to dry overnight under vacuum before experimentation. A sample size of 12 was used.

Statistical analysis

Statistical analysis was performed using Minitab software (Minitab, Version 15; Minitab Inc, Pennsylvania, USA). Values are expressed as mean ± standard error. Statistical comparisons were carried out by multiple analyses of variance (ANOVA) or by student t-test where appropriate. Randomized independent sampling was assumed and statistical significance was set at p < 0.05. All interaction terms were treated as fixed terms. Sample sizes varied between 3 and 12, as indicated per experiment.

Results

Design of EPD device

A 10 × 10 × 25 cm box was constructed out of chemically welded acrylic as a means of holding the deposition solution. The 3 × 8 × 0.1 cm rectangular metallic substrate was submerged in the deposition solution and was held in place via a simple holding device that was also constructed out of chemically welded acrylic. To keep the substrate mechanically stable, the 3 cm side of the substrate was pinched between two surfaces whose widths were 0.3 cm. The area exposed to the deposition solution was therefore 43.2 cm². An electric field was supplied via an Abra AB-3000 (Abra, Champlain, New York, USA) voltage source which remained at a constant voltage of 10.0 V throughout the experiment. Two rectangular counter-electrodes were placed parallel 4 cm from either side of the substrate to obtain a uniform electric field of 2.5 V/cm, which was sufficient to induce alginate deposition. A long thin metallic strip that ran along the side of the substrate holder was used as the contact surface between the voltage source and the substrate. It was positioned such that it was not exposed to the solution. An overview of the device design is shown in Figure 1. In preliminary experiments, it was observed that highly concentrated solutions formed deposits with poor thickness uniformity, so the deposition concentration was kept at 0.4% for all experiments in this paper.

Alginate deposition rates

The EPD device was used to deposit alginate on a flat geometry. The device was designed to be flexible such that other hydrogels can easily be substituted in the deposition solution. Moreover, a co-deposition can also be performed with another particle. The device is an excellent tool for modeling the deposition rates as the solution is geometrically constrained to the same cross-section as the substrate, ensuring the electric field is approximately constant over the substrate surface. Moreover, a large volume of deposition solution can be held by the device, allowing for a constant-concentration approximation.
assumption to be taken, although it must be supported with rigorous calculations based on the mass of the deposit layer formed. The mass of the alginate films increased approximately linearly for the first few time points, but over time the deposition rates decreased. The surface was smooth for low deposition times, but dimples and variations in colour concentration appeared as deposition time approached 10 minutes.

Figure 2 shows the alginate film mass as a function of time. For the first 180 seconds, the LV and MV grade alginates deposited at the same rate which was slightly below that of the HV grade alginate. By 10 minutes, the HV gel had the lowest mass, implying that the HV grade alginate deposition rate was most severely slowed down in the later stage of the deposition. By the end of the deposition, the LV and MV gels had different masses, with the MV being the higher of the two. All three gels were found to be significantly different from one another with p-values <0.005.

Cross linking mass reduction

The cross-linked gel mass was calculated as a percent of the initial mass and is shown in Figure 3. The MV and HV gels had similar changes in mass (p-value = 0.508) with a reduction of 72.3 ± 1.2%. The LV gel mass decreased the most with a reduction of 49.1 ± 1.8%.

Gel description

All un-cross-linked gels were opaque, with a yellow tint. The side of the gel that was exposed to the deposition solution had bumps, while the substrate side of the gel was flat. The opacity of the un-cross-linked gels decreased with viscosity. The cross-linked gels were more opaque than their un-cross-linked counterparts. Images of gels obtained after 5 minute depositions are shown in Figure 4. A water displacement test was performed on the gels and showed they all had a density of approximately 1.0 g/cm³.

Effect of lyophilization on alginate gels mass

The dry alginate mass was calculated as a percent of the post-deposit gel mass. The dry alginate contents were statistically similar (p-values > 0.266) for all three gels with dry mass fractions of 2.85± 0.46% show in Figure 5. When the gels were cross-linked, their water contents were reduced. The HV gels had similar dry masses (p = 0.839) of 7.07 ± 0.46%, while the LV gel has a dry mass of 5.59 ± 0.07%.

Molecular weight through gel permeation chromatography

The values of M sweetness Mw and Mz are shown in Table 1. It was shown that for all measurements except Mn, the molecular weight increased with viscosity grade. This indicates that although the majority of the mass of the HV alginate was found in the higher molecular weight range, there was a larger presence of smaller molecules. Increased molecular weights are associated with higher alginate viscosity [29,30], and the relation between the two have been discussed in literature [31,32].
Dried Alginate Gels’ Contact Angles

Contact angles are expressed from the water side of the droplet, so angles <90° are hydrophilic. All gels had statistically similar (p-values > 0.193) contact angles of 47.4 ± 1.1° except for the cross-linked HV gel whose contact angle was 37.2 ± 6.38° as shown in Figure 6. As the dried gel is both highly hydrophilic and porous, droplets would absorb quickly, which warped the surface of the dried gels. Contact angles were therefore taken quickly after droplets were deposited before warping occurred.

Discussion

EPD Device

EPD devices commonly employ large baths of deposition solution while completely neglecting the effects of solution geometry on the local electric field strength. For substances that deposit relatively much quicker than ceramics such as hydrogels, this means that an uneven deposition can become very pronounced, which can skew the results for modeling purposes. Boccaccini et al. for example describe an EPD device in which the substrate was held in an excessively large solution bath [2]. Although such a container is flexible in that unspecific substrate geometries can be used, it is not ideal for modeling the deposition rates. Non-uniform coatings may also have played a role in the non-linear correlation between chitosan concentration and deposition rate [33]. In another study, quartz crystal microbalance method of measuring deposit mass was used to yields significantly more time points [27], but it cannot be used for extensive deposition times where the deposit resistance becomes significant.

The device presented in this study overcomes the above mentioned obstacles. The container shape forces the deposition solution into a geometry such that the electric fields are parallel, ensuring the electric field strength is uniform over the surface of the substrate. The ability to weigh deposits after extended deposition times allowed for the characterization of gel deposition rate well beyond the non-linear range which has not been attempted before for alginate. The device could easily be used with other charged polymers, hydrogels, ceramics and nanoparticles as well as other suspension mediums such as ethanol and other organic solvents as long as the electric field strength and direction is controlled appropriately.

Alginate Deposition Rates

EPD rates were first modeled by Hamaker [34] who obtained the following relation describing the process:

$$\frac{dm}{dt} = \mu_e C S E$$

where $\mu_e$ is the electrophoretic mobility [cm²/Vs], C is the concentration [g/cm³], S is the surface area of the substrate [cm²], E is the electric field strength [V/cm].

Figure 4: Photographs of Alginate Gels. (a-c) Un-cross-linked and (d-f) cross linked (a, d) low viscosity, (b, e) medium viscosity and (c, f) high viscosity grade gels formed through electrophoretic deposition are shown.
The alginate deposition curves obtained from our EPD device were fitted to the above Sarkar equation using MatLab R2010a (MathWorks, Natick, Massachusetts, USA) cf tool function and the results and the relation for deposit mass with p-values < 0.033. Error bars are in S.E.M., n = 3.

### Table 1: Viscosities of Deposition Solutions- The molecular weights of the three sample algelates were obtained courtesy of the Laboratoire de Caractérisation des Matériaux Polymères au Département de chimie at L’Université de Montreal. From $M_p$, $[n]$ was calculated using the Mark-Houwink-Sakurada equation [32], which allowed for the calculation of the critical concentration C*. Using the viscosities of the 2% solutions which were provided by the manufacturers, the 0.4% viscosities were then calculated.

| Alginate Grade | $M_p$ [kDa] | $M_x$ [kDa] | $M_z$ [kDa] | [η] [ml/g] | [%] | [cP] | [cP] |
|----------------|-------------|-------------|-------------|-----------|-----|------|------|
| 0.4% M. pyrifera LV | 196 | 643 | 1,652 | 208 | 1.92 | 250 | 24.4 |
| 0.4% M. pyrifera MV | 196 | 779 | 2,055 | 236 | 1.70 | 3500 | 269 |
| 0.4% M. pyrifera HV | 178 | 981 | 2,446 | 275 | 1.46 | 14,000 | 865 |

To explain this phenomenon, longer depositions are modelled either as constant current or constant voltage conditions. These are further classified into the two scenarios where the deposition solution concentration is or isn’t kept constant. Under constant voltage/constant concentration conditions, the buildup of a resistive layer of deposit slows down the deposition rate. Although this relationship was true for short deposition times, the viscosity grade is a good indicator of the friction forces resisting particle motion towards the substrate, the electrolytic force is much more complex, and depends on particle charge, inter-particle interactions, and local pH which vary along the distance between the electrodes [35]. The viscosity of the HV gel indicated that the friction force would have been higher during EPD compared to the other two gels. The HV gel deposition rate slowed down the most throughout the run due its larger $R_f$.

### Cross-linking

The MV and HV gels had a larger mass reduction following cross-linking.

![Figure 5: Effect of Viscosity Grade on Dry Alginate Mass- Alginate gels were lyophilized for 2 days either before or after cross-linking. The dry alginate masses of the un-cross-linked (white) and cross-linked (grey) gels are represented above. Un-cross-linked gels had statistically similar dry masses (p-values > 0.266) of 2.85 ± 0.46%. Cross-linking increased all of the dry masses with p-values < 0.033. Error bars are in S.E.M., n = 3.](image)

### Table 2: Deposition Rate Modelling Parameters: Values of $\alpha$ and $\beta$ were obtained by curve-fitting the mass vs. time curves shown in Figure 2 with the Sarkar equation for constant-voltage/constant-concentration deposit mass. From this, the electrophoretic mobility ($\mu_e$) and deposit-solution resistivity ratios ($R_f$) were obtained.

| Alginate Grade | $\alpha$ [g/cm³] | $\beta$ [g/cm²] | $\mu_e$ [cm²/Vs] | $R_f$ [unitless] |
|----------------|-----------------|-----------------|-----------------|-----------------|
| 0.4% M. pyrifera LV | 0.2302 | 0.02634 | 0.0610 | 21.0 |
| 0.4% M. pyrifera MV | 0.1759 | 0.02524 | 0.0584 | 16.2 |
| 0.4% M. pyrifera HV | 0.5377 | 0.03927 | 0.0909 | 47.5 |

The first two terms of the Maclaurin series for the equation yields:

$$m = \beta \left(1 - \frac{2a\beta t}{\alpha}\right)$$

The electrophoretic mobility is an empirical value that results from the balance between the electrostatic forces and the friction forces exerted on the particle. Although the viscosity grade is a good indicator of the friction forces resisting particle motion towards the substrate, the electrostatic force is much more complex, and depends on particle charge, inter-particle interactions, and local pH which vary along the distance between the electrodes [35]. The viscosity of the HV gel indicated that the friction force would have been higher during EPD compared to the other two gels. The HV gel deposition rate slowed down the most throughout the run due its larger $R_f$. The electrophoretic mobility is an empirical value that results from the balance between the electrostatic forces and the friction forces exerted on the particle. Although the viscosity grade is a good indicator of the friction forces resisting particle motion towards the substrate, the electrostatic force is much more complex, and depends on particle charge, inter-particle interactions, and local pH which vary along the distance between the electrodes [35].

Figure 6: Effect of Alginate Viscosity Grade on Dry Gel Contact Angle-Gels from three different viscosity grades of alginate was produced using electrohydrodynamic deposition, and they were dried overnight in vacuum. The contact angles were then obtained by depositing a 0.25 μl drop of water on the dried gel surface. Un-cross-linked (white) and cross-linked (grey) gel contact angles are represented above. All gels had similar contact angle (p-values > 0.193) of 47.4 ± 1.1° except for the cross-linked HV grade alginate (p-value < 0.001) that had a contact angle of 37.2 ± 6.3°. Error bars are in S.E.M., n = 12.

Cross-linking

The MV and HV gels had a larger mass reduction following cross-linking.

In many EPD processes including those involving ceramics, the solute is the only species deposited on the substrate. In the case of hydrogels, however, the solvent also forms part of the deposited mass. It is therefore more practical to model the deposition rate in terms of the total mass of the deposited hydrogel and not just the polymer species. The alginate deposition curves obtained from our EPD device were fit to the above Sarkar equation using MatLab R2010a (MathWorks, Natick, Massachusetts, USA) cf tool function and the results and the relation for deposit mass with p-values < 0.033. Error bars are in S.E.M., n = 3.

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To explain this phenomenon, longer depositions are modelled either as constant current or constant voltage conditions. These are further classified into the two scenarios where the deposition solution concentration is or isn’t kept constant. Under constant voltage/constant concentration conditions, the buildup of a resistive layer of deposit slows down the deposition rate with time. Sarkar and Nicholson PS used the Hamaker equation to obtain the relation for deposit mass under these conditions [9]:

$$m = \frac{1}{\alpha} \left(1 + \sqrt{2\alpha 2\beta t + 1}\right)$$

$$\alpha = \frac{2(R_f - 1)}{L p S}$$

$$\beta = \mu, SCE$$

Where L is the distance between electrodes [cm], $R_f$ is the ratio of deposit resistivity to the suspension resistivity, and $\rho$ is the density of the deposit [g/cm³].

In many EPD processes including those involving ceramics, the solute is the only species deposited on the substrate. In the case of hydrogels, however, the solvent also forms part of the deposited mass. It is therefore more practical to model the deposition rate in terms of the total mass of the deposited hydrogel and not just the polymer species. The alginate deposition curves obtained from our EPD device were fit to the above Sarkar equation using MatLab R2010a (MathWorks, Natick, Massachusetts, USA) cf tool function and the results and the relation for deposit mass with p-values < 0.033. Error bars are in S.E.M., n = 3.
linking than did the LV gel. This is because the MV and HV alginates particles were larger, offering more sites for Ca\textsuperscript{2+} particle bridging. This resulted in a higher degree of cross-linking, and therefore a reduction in swelling properties. In literature, dry alginlate and alginlate microcapsules were also shown to have changes in mass increase with viscosity grade when they were moved from cross-linking solutions to water [22,36,37]. When alginlate microcapsules produced through an emulsion technique were placed in water [38], a slightly higher change in mass was observed compared gels produced using EDP subjected to a cross-linking solution.

All gels had approximately the same density so the change in volume was the same as the change in mass. Gel opacity was caused by micro-bubbles formed at the cathode. As bubble transportation from the cathode to the anode increased as viscosity decreased, it follows that opacity also increased as viscosity grade decreased. Gels were qualitatively observed to have improved mechanical properties following cross-linking. This is especially true for the LV grade alginlate. Future works should focus on a qualitative assessment of the gel.

**Contact angle**

The HV gels contact angle following cross-linking was likely smaller due to Ca\textsuperscript{2+} uptake. All surfaces were shown to be highly hydrophilic, with contact angles below 47.4°C. Hydrophilic materials are ideal for mammalian cell cultures [39] due to an uptake in adhesive proteins [40-42]. As hydrophobic materials are commonly antithrombotic and resistant to fibrin sheath formation [43], the gels may have potential in tissue engineering and implant coatings applications due to their increase biocompatibility. EDP would be especially useful as it would be possible to coat arbitrarily shaped tissue engineering scaffolds, so long as they are conductive. The hydrophilic surface is also ideal for immobilizing hydrophilic proteins.

**Viscosity calculations from molecular weights**

It was shown that cross-linking reduced the mass for the MV and HV grade alginates to a greater extent than it did for the LV grade alginlate. This is in accordance with previous studies that have shown a greater degree of cross-linking in alginates of higher molecular weight [44]; however it may also be due to differences in the fraction of G blocks in the polymer, which also have a higher affinity for Ca\textsuperscript{2+} ions. The viscosity grade increased with M\textsubscript{w} as shown in previous studies [19]. The loss of mass that occurred during cross-linking was least extreme in the HV gels, which resulted in them having the smallest change in water mass fraction after cross-linking out of the three gels.

The viscosities of the solutions used in the experiments were approximated using the relation obtained by Morris et al [31] whereby the concentration dependent viscosity of a random coil polysaccharide approximated using the relation obtained by Morris et al [31] whereby mass fraction after cross-linking out of the three gels.

All gels had approximately the same density so the change in volume was the same as the change in mass. Gel opacity was caused by micro-bubbles formed at the cathode. As bubble transportation from the cathode to the anode increased as viscosity decreased, it follows that opacity also increased as viscosity grade decreased. Gels were qualitatively observed to have improved mechanical properties following cross-linking. This is especially true for the LV grade alginate. Future works should focus on a qualitative assessment of the gel.

**Conclusion**

A novel EDP device was designed to effectively deposit alginlate on a flat substrate. Alginlate films were then produced on thin steel rectangles using three alginates of different viscosity grades. The deposition mass was quantified and was used to show that the process could be modeled using the Sarkar equation for a constant voltage/constant concentration EDP. A change in mass was observed following cross-linking using CaCl\textsubscript{2} solution, with the LV grade alginate having the smallest change in mass. The dry alginate content was similar for all samples before cross-linking, but it was lower for the cross-linked LV grade alginate than for the other two. Although the viscosity grade affected the deposition rate, the Sarkar model was still accurate as long as appropriate μ\textsubscript{e} and R\textsubscript{f} values were used. Although results indicated that many properties varied with viscosity grade, it is possible that properties may become more extreme with a larger range of grades. These results will be useful in the design and manufacturing of devices that incorporate EDP of sodium alginlate. Moreover, the methods outlined in this paper can be extrapolated to model other and understand hydrogel systems.

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