Rheological Investigation of Thermoresponsive Alginate-Methylcellulose Gels for Epidermal Growth Factor Formulation

Olivia Eskens, Gianna Villani and Samiul Amin *

Laboratory of Cosmetics and Pharmaceuticals, Department of Chemical Engineering, Manhattan College, Riverdale, New York, NY 10471, USA; oeskens01@manhattan.edu (O.E.); gvillani01@manhattan.edu (G.V.)
* Correspondence: samin01@manhattan.edu

Abstract: Epidermal growth factors (EGF) serve as promising candidates for skin regeneration and rejuvenation products, but their instability hinders them from widespread use. Protective immobilization and directed release can be achieved through implementing a hydrogel delivery system. Alginate and methylcellulose are both natural polymers offering biocompatibility and environmental sensitivity. This blended gel system was investigated rheologically to understand its performance in topical applications. Alginate and methylcellulose were found to form a synergistic gel system that resulted in superior viscosity and thermoresponsiveness compared to the individual components. Increasing methylcellulose concentration directly enhanced gel elasticity, and higher viscosities provided better thermal protection of EGF. The addition of EGF at 3.33 mg/mL resulted in a decrease of viscosity but an increase in viscoelastic modulus. EGF concentration also played a large role in shear viscosity and thermoresponsiveness of the ternary system. An alginate-methylcellulose system presents promising rheological tunability, which may provide EGF thermal protection in a topical delivery format.

Keywords: epidermal growth factor; rheology; thermal response; smart polymers; skin regeneration; topical delivery; hydrogel; alginate; methylcellulose

1. Introduction

The epidermal growth factor (EGF) is one of the earliest known polypeptide growth factors and the originator of the EGF-like protein family. This class of peptides has highly similar structural and functional characteristics, promotes mitogenic activity, and typically uses the same receptors for response triggering [1]. EGF directly affects collagen, elastin, and extracellular matrix biosynthesis, making it a prime candidate for skin regeneration and rejuvenation. These peptides also have characteristically short half-lives and low intercellular diffusion rates, allowing them to operate locally in the body but making them challenging to formulate with. EGF possesses a variety of functional groups and a threedimensional conformation allowing degradation via chemical and physical pathways such as protein denaturation, aggregation, hydrolysis of peptide bonds, or oxidation of amino acid side chains [2]. The integrity of growth factors is easily compromised, especially in prolonged storage conditions, by fluctuations in pH, temperature, or complex electrostatic and hydrophobic interactions [3]. EGF has an optimal pH stability range from 6.0 to 8.0 with an isoelectric point at 4.6, and an unfolding onset around 40 °C with a transition midpoint at 55.5 °C [4,5]. Implementing delivery systems for EGF formulation has the potential to reduce the peptide’s instability through diffusion-limiting immobilization and protective encasing [6].

Polymeric biomatrix characteristics such as water content or crosslink density can be tailored to optimize the diffusion-controlled EGF retention and release mechanisms. The physicochemical crosslinking of polymers also leaves them especially responsive to
environmental changes. In this study, we attempt to tune a hydrogel delivery vehicle with thermoresponsiveness for EGF formulation. The target of the gel system is to effectively immobilize EGF at storage conditions and evenly distribute EGF through thermal and shear-induced gel degradation upon topical application. Hydrogels provide excellent flexibility and hydration for skin compatibility, while extracellular matrix-inspired delivery materials utilize the natural growth factor affinity and attachment techniques to mimic dermal release. For this reason, alginate is one of the components of the polymeric network. Additionally, alginate has previously shown an increase in EGF encapsulation efficiency as well as inherent regenerative properties [7]. Alginates transform to match the functions of skin cells and organs, which eventually leads to healing of the affected area on the body [8]. Sodium alginate is an anionic, hydrophilic polysaccharide that is extracted from the cell walls of brown algae and various strains of bacteria. The molecular structure of sodium alginate is a linear alginic acid copolymer with linked 1-4 glucosidic bonds. These glucosidic bonds are formed by the homopolymeric blocks of β-D-mannuronic acid and α-L-guluronic acid, outlined in Figure 1. Alginate that contains a high level of G-block content results in stiff and brittle gels that tend to be more stable, while high M-block content leads to soft, elastic gels with high water adsorption and ion exchange [8]. This is due to the attractive preferential forces of ions to the extended G-block residues within the alginate structure [9]. The properties of alginate gels can be adjusted with molecular weight, concentration, G-block distribution, cation availability, and constituent interactions [8].

![Figure 1. The chemical structure of alginate.](image)

Methylcellulose is the other component selected for the interpenetrated polymer network due to its thermoresponsive behavior. Methylcellulose is a natural, heterogeneous polymer that forms reversible gels upon heating, and its chemical structure is outlined in Figure 2. The highly substituted hydrophobic groups within methylcellulose form attractive complexes at increased temperatures, causing gelation [10]. At semi-dilute conditions, as seen in our system, the viscosity of methylcellulose solutions decreases up to a critical value then increases as a turbid gel develops [10,11]. Mixing alginate with methylcellulose creates a complex gel system where hydrophobic interaction, hydrogen bond formation between carboxyl and hydroxy groups, and ionic crosslinking are synchronous [12]. The mild gelling conditions of both polymers, along with their immunogenic nature, make them ideal candidates for the preservation of sensitive active pharmaceutical components [8,12]. This study aims to characterize the thermo-rheological behavior of the fluid gel system and the effect of EGF when introduced into the polymeric network.
Figure 2. The chemical structure of methylcellulose.

2. Materials and Methods

Alginic acid sodium salt from brown algae with medium viscosity was obtained from Sigma-Aldrich Inc. (St. Louis, MO, USA). Methylcellulose from Alfa Aesar (Ward Hill, MA, USA) with a defined 2% aqueous solution viscosity of 400 cP at 20 °C was used. Alginate and methylcellulose were simultaneously stirred into a solution of deionized water at room temperature. To investigate composition-dependent gel properties, first, the alginate concentration was held constant at 1% w/v while methylcellulose concentration varied between 1%, 1.5%, and 2% w/v. Epidermal growth factor was obtained from Skin Actives Scientific. The peptide gels were originally formulated at 3.33 mg/mL and incorporated prior to polymeric dissolving. After the initial investigation, the alginate concentration was varied while methylcellulose and peptide concentrations were held constant at 2% w/v and 3.33 mg/mL, respectively. Finally, the peptide concentration was varied in the 1% alginate–2% methylcellulose gel system with additional concentrations of 1.67 and 6.67 mg/mL. All solutions maintained a near-neutral pH. The viscosity, complex moduli, and thermoresponsiveness of the resulting gel systems were investigated.

The Discovery Hybrid Rheometer (DHR-3) from TA Instruments was employed to determine the rheological properties of the alginate-methylcellulose systems both with and without EGF. A stainless steel, 40 mm cone plate with a 2-degree angle was used to conduct all rheological experiments on the temperature-controlled Peltier plate. Flow sweeps were carried out between the shear rate range of 0.1 to 5000 s\(^{-1}\). This encompasses storage, spreading, pumping, and rubbing processes during product utilization. An amplitude sweep was carried out over 0.01% to 50% oscillation strain with a constant angular frequency of 10 rad s\(^{-1}\) on each gel system to define their individual linear viscoelastic regions. Frequency sweeps were conducted within the established linear viscoelastic region with angular frequency ranging from 0.1 to 100 rad s\(^{-1}\). All of these outlined experiments were performed isothermally at 25 °C, and measurements were taken under steady-state sensing conditions. Temperature sweeps evaluated the thermosensitivity as the gels approach and surpass the skin’s surface temperature of 37.5 °C. A constant shear rate of 10 s\(^{-1}\) was applied to the sample, and the temperature was increased by 1°C per minute between 25 °C and 50 °C. All experiments were run a minimum of twice to ensure reproducibility. The data presented in this article are the mean values of each sample’s various trials, and across all experiment and sample types, a relative standard deviation of 10% or less was observed. Error bars for individual values were excluded in the following graphs for the sake of clarity.

3. Results and Discussion

The deformation and flow behavior of the fluid gel system is important to characterize the storage and release mechanisms of the EGF-loaded product when temperature and shear stressors are applied. Peptide solutions are often stored at cool temperatures to prolong product life, and a thick interpenetrated polymer network should further limit EGF deactivation. As the product is topically applied, temperature increases to match the body, and high shear stresses are imposed, resulting in structural breakdown and pseudoplastic behavior. We aimed to introduce the impact of gel composition on the structure-property relationship of the ternary system.
3.1. Dynamic Viscosity

The viscosity profile of the fluid gel is linked to the polymeric network and strength of constituent interactions. Figure 3 explores the influence of methylcellulose concentration on the shear viscosity. The flow behavior of sodium alginate-methylcellulose systems has been previously characterized best by the non-Newtonian Hershel–Buckley fluid model [13]. The dominant solution interactions of alginate are charge repulsions between dissociated carboxyl groups and the formation of hydrogen bonds between carboxylic acids and ionized carboxyl groups. Methylcellulose solution interactions rely heavily on hydrophobic chain grouping. Both alginate and methylcellulose solutions have a distinctive pseudoplastic behavior at neutral pH. Some hydrophobic sections appear in alginate chains upon protonation of dissociated carboxyl groups [13]. This gives rise to an increase in hydrophobic association between alginate and methylcellulose in the interpenetrated polymer network. The result is a synergistic combination that has a higher viscosity than either individual component while shear-thinning is preserved, and this trend is also seen in an alginate-methylcellulose mixture studied by Mehmandoost et al. [13]. This is not uncommon as enhanced stability of physical and mechanical properties of biopolymer blends has been appreciably reported [14–18].

![Figure 3](image-url)

**Figure 3.** The viscosity vs. shear rate behavior of alginate (blue), methylcellulose (yellow), and mixtures of the two (green) with varying methylcellulose concentration (% w/v). The circular symbols appearing in the plots represent the mean-values of discrete measurements evenly acquired at logarithmic intervals under steady-state conditions, while the continuous lines are guides for the eye. MC: methylcellulose; Alg: alginate.

The impact of EGF addition on the shear viscosity of the mixtures with varying methylcellulose concentration can be observed in Figure 4. When EGF was introduced into the system, there was a marginal viscosity drop across all samples. The low molecular weight polypeptide may disrupt the polymer structural entanglement slightly, but the synergistic and pseudoplastic nature of the fluid gel was still retained. It is expected that the high viscosity immobilizes EGF while topical application begins to breakdown the gel structure. At neutral pH, EGF carries a negative charge and may demonstrate repulsive interactions with the anionic alginate chain. EGF is also minorly composed of hydrophobic residues, which possibly complicates the hydrophobic relationship between the long-chain polymers.
Figure 4. Shear viscosity behavior of alginate-methylcellulose mixtures (green) with varying methylcellulose concentrations (\% w/v) and epidermal growth factor (EGF) addition at 3.33 mg/mL (red). The circular symbols appearing in the plots represent the mean values of discrete measurements evenly acquired at logarithmic intervals under steady-state conditions, while the continuous lines are guides for the eye.

3.2. Viscoelastic Modulus

The dynamic modulus further explores the system interactions and performance by evaluating gel stiffness. The storage modulus (G’) of pure alginate is consistently lower than the loss modulus (G’’), and the same development is seen in Figure 5 [19]. While the viscous property of alginate remains dominant, the difference between the two moduli decreased at higher angular frequencies revealing a decrease in phase angle and a fluid-like viscoelastic behavior. On the other hand, methylcellulose viscoelastic behavior resembled more of an elastic gel system where G’ is typically greater than G’’, and the change in phase angle is smaller over the range of angular frequency. The viscoelastic transition of the alginate-methylcellulose systems is better visualized in Figure 5c. The G’/G’’ crossover took place at higher angular frequencies as methylcellulose concentration increased. The elastic component of the system took stronger dominance as methylcellulose concentration increased, and gel stiffness was enhanced. While the variance of total gel concentration between samples generates some inconclusively, this outcome is similarly embodied by an alginate-carboxymethylcellulose system, as studied by Zheng et al. [19].

Recalling that EGF caused a slight decrease in shear viscosity behavior, the impact on dynamic modulus should give more insight into the molecular interactions happening in the system. The influence of EGF addition on each gel system with varying methylcellulose concentration is explored in Figure 6. The strength of the gel does not appear to be jeopardized, suggesting a more complex interrelationship. It was observed that the change in phase angle decreases with increasing methylcellulose concentration, but the viscoelastic transition is not as dependent on methylcellulose concentration when EGF is in the system. In every instance measured, EGF increased gel stiffness signifying that the loss in viscosity is not necessarily due to a loss of structure. This is conversely related to the impact of silicone in a silicated hydroxypropylmethylcellulose-alginate gel network that hindered effective alginate chain interactions and ultimately reduced gel stiffness [18]. Although elastic modulus increases with the addition of EGF, the relaxation times shift to higher frequencies meaning shorter relaxation times. This may signal a new network structure forming between EGF and methylcellulose due to hydrophobic interaction. The complexity of the ternary interdependence needs to be more substantially defined in order to fully interpret viscoelastic properties.
Recalling that EGF caused a slight decrease in shear viscosity behavior, the impact on dynamic modulus should give more insight into the molecular interactions happening in the system. The influence of EGF addition on each gel system with varying methylcellulose concentration is explored in Figure 6. The strength of the gel does not appear to be jeopardized, suggesting a more complex interrelationship. It was observed that the change in phase angle decreases with increasing methylcellulose concentration, but the viscoelastic transition is not as dependent on methylcellulose concentration when EGF is in the system. In every instance measured, EGF increased gel stiffness signifying that the loss in viscosity is not necessarily due to a loss of structure. This is conversely related to the impact of silicone in a silicated hydroxypropylmethylcellulose-alginate gel network that hindered effective alginate chain interactions and ultimately reduced gel stiffness [18]. Although elastic modulus increases with the addition of EGF, the relaxation times shift to higher frequencies meaning shorter relaxation times. This may signal a new network structure forming between EGF and methylcellulose due to hydrophobic interaction. The complexity of the ternary interdependence needs to be more substantially defined in order to fully interpret viscoelastic properties.

**Figure 5.** Viscoelastic moduli $G'$ (●) and $G''$ (▲) of the resulting gel systems: (a) 1% w/v alginate (blue), 1% w/v methylcellulose (yellow), and the mixture of the two (green); (b) 1% w/v alginate (blue), 2% w/v methylcellulose (yellow), and the mixture of the two (green); (c) Alginate-methylcellulose mixtures with varying methylcellulose concentration (w/v). The circular and triangular symbols appearing in the plots represent the mean-values of discrete measurements evenly acquired at logarithmic intervals under steady-state conditions, while the continuous lines are guides for the eye. $G$: storage modulus; $G'':$ loss modulus.
Figure 6. The impact of 3.33 mg/mL epidermal growth factor addition (red) on the viscoelastic moduli $G'$ (●) and $G''$ (▲) of various alginate-methylcellulose mixtures (green) with increasing methylcellulose concentration (w/v): (a) 1% alginate–1% methylcellulose; (b) 1% alginate–1.5% methylcellulose; (c) 1% alginate–2% methylcellulose. The circular and triangular symbols appearing in the plots represent the mean-values of discrete measurements evenly acquired at logarithmic intervals under steady-state conditions, while the continuous lines are guides for the eye.
3.3. Thermoresponsivity

It is known that composition affects the initial thermal transitions and gelling temperatures of compound gel systems [20]. Figure 7a shows the impact of polymer blending and methylcellulose concentration on thermoresponsiveness. As expected, pure alginate showed a negligible reaction to increased temperature, while methylcellulose decreased in viscosity until a critical temperature is met. The critical temperature of pure methylcellulose was not significantly influenced by the concentration range studied. As the polymers were mixed together, thermoresponsiveness was enhanced. The hydrophobic gelling mechanism of methylcellulose is suspected of intensifying with increasing concentration, and hydrophobic interactions from alginate enhance the thermal transition. The thermal reduction of viscosity reveals a dual-mechanistic breakdown of the prepared gel network during topical application. When EGF was introduced to these systems, new gel characteristics were observed. Figure 7b shows a stronger decrease in viscosity before the critical gelling temperature was met. This may be attributed to EGF thermal instability and the consequential structural changes impacting the polymeric network. Introduction of EGF generally increased the critical temperature in the mixtures studied and proposes that the largely hydrophilic structure of EGF somewhat obstructs the hydrophobic gelling procedure.

![Graph](image)

**Figure 7.** The response of gel systems with varying methylcellulose concentration (w/w) as temperature increased by 1 °C per minute: (a) Alginate (blue), methylcellulose (yellow), and the resulting mixtures of the two (green); (b) The impact of 3.33 mg/mL epidermal growth factor addition (red) on alginate-methylcellulose mixtures (green). Viscosity values were continuously acquired as a function of increasing temperature.

The influence of alginate on the mixture was investigated with viscosity versus temperature tests visualized in Figure 8a. The shear viscosity of the mixture seemed to be more strongly dependent on the alginate content. This could be due to the amplification of supportive electrostatic interactions but also increased entanglement from the larger overall polymer content. An effective thermal decrease in viscosity is observed in these samples, but a critical temperature is not met in the testing range. The ratio of the components manipulates the onset of hydrophobic complexion. The pH-dependent, anionic nature of alginate makes a good delivery system for cationic drugs through electrostatic interactions [21]. Sodium alginate interacted with lactoferrin through electrostatic attraction and increased the heat stability of the protein [22]. It is important to consider the lactoferrin isoelectric point of 8.8, giving it a net positive charge in the neutral alginate
gel, whereas EGF has an acidic isoelectric point. Interestingly, vascular endothelial growth factor encapsulation and release behavior were not affected by alginate concentration or G-block content even though it also has a basic isoelectric point of 8.5 [23]. Bovine serum albumin has a similar isoelectric point to EGF, and its release from a comparable hydrogel network at a pH of 7.4 was strongly dependent on alginate concentration [12]. Additionally, electrostatic behavior within alginate is not influenced by heat treatment [22]. In Figure 8b, it can be clearly seen that the critical temperature is increased with higher net-polymer and alginate concentrations. There is less irregularity as the viscosity of these EGF-loaded gels minimizes, indicating better protective capability. The viscosity enhancement may provide a more immobilized structure, and alginate electrostatic behavior and hydrogen bonding could increase the EGF affinity to the gel analogous to findings with other proteins.

Figure 8. The response of gel systems with varying alginate concentration (w/v) as temperature increased by 1 °C per minute: (a) Alginate (blue), methylcellulose (yellow), and the resulting mixtures of the two (green); (b) The impact of 3.33 mg/mL epidermal growth factor addition (red) on alginate-methylcellulose mixtures (green). Viscosity values were continuously acquired as a function of increasing temperature.
Lastly, the impact of EGF concentration on thermoresponsiveness was studied from Figure 9 for the 1% alginate–2% methylcellulose system. As EGF concentration increased, the critical temperature of the system decreased, signaling greater thermal instabilities. Similarly, higher concentrations of growth factors lead to lower encapsulation yields in other alginate delivery systems [8]. It is suspected that at higher EGF concentrations, the protective capability of the gel network is insufficient. With this comes an implied pivotal ratio between EGF and polymer content where instabilities are effectively minimized. The sample containing the lowest amount of protein demonstrated additional synergy as the shear viscosity surpassed that of the original alginate-methylcellulose system.

![Figure 9](image.jpg)

**Figure 9.** The effect of epidermal growth factor concentration (red) on the viscosity of 1% w/v alginate–2% w/v methylcellulose gel mixture (green) when temperature increased by 1 °C per minute. Viscosity values were continuously acquired as a function of increasing temperature.

### 4. Conclusions

There is a wide range of prospective research to further define the structure-property relationship of this alginate-methylcellulose-EGF network. Alginate and methylcellulose were found to form a synergistic gel system that resulted in superior viscosity and thermoresponsiveness compared to the individual components due to an increase in hydrophobic association in the interpenetrated polymer network. The stiffness and elasticity of the gel mixture were directly related to methylcellulose concentration. The addition of EGF resulted in a decrease in viscosity but an increase in viscoelastic modulus. A new hydrophobically-associated network is suggested to form with EGF incorporation as shorter relaxation times were observed in the ternary system. EGF concentration also played a large role in thermoresponsiveness of the hydrogel mixtures attributed to its inherent thermal instability. Overall, this system presents a great opportunity for a highly tunable and effective delivery vehicle for EGF with thermoresponsive behavior. The protectivity of the gel is suspected to be dependent on the ratio of polymer to the peptide as well as individual polymer composition, but this aspect requires further investigation.

**Author Contributions:** Conceptualization and methodology, S.A.; investigation and validation, O.E. and G.V.; data curation and visualization, O.E.; supervision and analysis, S.A.; writing, O.E. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.
Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ebner, R.; Derynck, R. Epidermal growth factor and transforming growth factor-α: Differential intracellular routing and processing of ligand-receptor complexes. Cell Regul. 1991, 2, 599–612. [CrossRef] [PubMed]

2. Senderoff, R.I.; Wootton, S.C.; Docter, A.M.; Chen, T.M.; Giordani, A.B.; Julian, T.N.; Radebaugh, G.W. Aqueous stability of human epidermal growth factor 1-48. Pharm. Res. 1994, 11, 1712–1720. [CrossRef] [PubMed]

3. Arakawa, T.; Prestrelski, S.J.; Kenney, W.C.; Carpenter, J.F. Factors affecting short-term and long-term stabilities of proteins. Adv. Drug Deliv. Rev. 2011, 46, 307–326. [CrossRef]

4. Santana-Milán, H.; González, Y.; Campana, P.T.; Noda, J.; Amarantes, O.; Itri, R.; Beldarrain, A.; Páez, R. Screening for stability and compatibility conditions of recombinant human epidermal growth factor for parenteral formulation: Effect of pH, buffers, and excipients. Int. J. Pharm. 2013, 452, 52–62. [CrossRef]

5. Yang, C.; Wu, P.; Huang, Y.; Tsai, Y. A new approach for determining the stability of recombinant human epidermal growth factor by thermal Fourier transform infrared (FTIR) microspectroscopy. J. Biomol. Struct. Dyn. 2004, 22, 101–110. [CrossRef]

6. Eskens, O.; Amin, S. Challenges and Effective Routes for Formulating and Delivery of Epidermal Growth Factors in Skin Care. Int. J. Cosmet. Sci. 2020. [CrossRef]

7. Gainza, G.; Aguirre, J.J.; Pedraz, J.L.; Hernández, R.M.; Igartua, M. rhEGF-loaded PLGA-alginate microspheres enhance the healing of full-thickness excisional wounds in diabetic Wistar rat. Eur. J. Pharm. Sci. 2013, 50, 243–252. [CrossRef]

8. Wawrzyńska, E.; Kubies, D. Alginate matrices for protein delivery—A short review. Physiol. Res. 2018, 67, 319–334. [CrossRef]

9. Fernández Farrés, I.; Norton, I.T. Formation kinetics and rheology of alginate fluid gels produced by in-situ calcium release. Food Hydrocoll. 2014, 40, 76–84. [CrossRef]

10. Desbrière, J.; Hirrien, M.; Ross-Murphy, S.B. Thermogelation of methylcellulose: Rheological considerations. Polymer 2000, 41, 2451–2461. [CrossRef]

11. Kundu, P.P.; Kundu, M. Effects of salts and surfactant and their doses on the gelation of extremely dilute solutions of methyl cellulose. Polymer 2001, 42, 2015–2020. [CrossRef]

12. Liang, H.F.; Hong, M.H.; Ho, R.M.; Chung, C.K.; Lin, Y.H.; Chen, C.H.; Sung, H.W. Novel method using a temperature-sensitive polymer (methylcellulose) to thermally gel aqueous alginate as a pH-sensitive hydrogel. Biomacromolecules 2004, 5, 1917–1925. [CrossRef] [PubMed]

13. Mehmandoost, F.; Hojjatoleslamy, M.; Keramat, J.; Behzadiya, A.; Shahbazpour, N. Effect of pH and calcium salt on rheological properties of sodium alginate -methyl cellulose mixtures. Int. J. Biosci. 2013, 3, 105–114. [CrossRef]

14. Sahana, T.G.; Rekha, P. Biopolymers: Applications in wound healing and skin tissue engineering. Mol. Biol. Rep. 2018, 45, 2857–2867. [CrossRef]

15. Turgeon, S.I.; Schmitt, C.; Sanchez, C. Protein–polysaccharide complexes and coacervates. Curr. Opin. Colloid Interface Sci. 2007, 12, 166–178. [CrossRef]

16. Sun, J.-Y.; Zhao, X.; Ileperuma, W.R.K.; Chaudhuri, O.; Oh, K.H.; Mooney, D.J.; Vlassak, J.J.; Suo, Z. Highly stretchable and tough hydrogels. Nature 2012, 489, 133–136. [CrossRef]

17. Darnell, M.C.; Sun, J.-Y.; Mehta, M.; Johnson, C.; Arany, P.R.; Suo, Z.; Shetye, S. Performance and biocompatibility of extremely tough alginate/polyacrylamide hydrogels. Biomaterials 2013, 34, 8042–8048. [CrossRef]

18. Viguier, A.; Boyer, C.; Chassenieux, C.; Benyahia, L.; Guicheux, J.; Weiss, P.; Rethore, G.; Nicolai, T. Interpenetrated Si-HPMC/alginate hydrogels as a potential scaffold for human tissue regeneration. J. Mater. Sci. Mater. Med. 2016, 27, 99. [CrossRef]

19. Zheng, J.; Zeng, R.; Zhang, F.; Kan, J. Effects of sodium carboxymethyl cellulose on rheological properties and gelation behaviors of sodium alginate induced calcium ions. LWT Food Sci. Technol. 2019, 103, 131–138. [CrossRef]

20. Soledad Lencina, M.M.; Rizzo, C.; Demitri, C.; Andreucetti, N.; Maffezzoli, A. Rheological analysis of thermo-responsive alginate/PNIPAAm graft copolymers synthesized by gamma radiation. Radiat. Phys. Chem. 2019, 156, 38–43. [CrossRef]

21. Sun, J.; Tan, H. Alginate-based biomaterials for regenerative medicine applications. Materials 2013, 6, 1285–1309. [CrossRef] [PubMed]

22. Li, Q.; Lan, H.; Zhao, Z. Protection effect of sodium alginate against heat-induced structural changes of lactoferrin molecules at neutral pH. LWT Food Sci. Technol. 2019, 99, 513–518. [CrossRef]

23. Gu, F.; Amsden, B.; Neufeld, R. Sustained delivery of vascular endothelial growth factor with alginate beads. J. Control Release 2004, 96, 463–472. [CrossRef] [PubMed]