# OPTIMIZING POLYSACCHARIDE-PROTEIN HYDROGELS FOR REGENERATIVE MEDICINE AND CELLULAR AGRICULTURE: A RHEOLOGICAL STUDY

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## ABSTRACT

This study explores the formulation and characterization of xanthan gum-alginate-gelatin (AXG) hydrogels for regenerative medicine and cellular agriculture applications. Three formulations - AXG-2:1, AXG-1:1, and AXG-1:2 - were tested, with varying ratios of alginate to xanthan gum. Filament extrusion tests showed that all formulations produced homogeneous filaments, with xanthan-rich hydrogels retaining shape better upon deposition. Tube inversion tests confirmed that none of the hydrogels flowed within 15 min, indicating good structural stability. Crosslinking assessments demonstrated that all formulations gelled within 30 min in a $CaCl_2$ solution. Rheological evaluations revealed that the n index was consistently below 1, indicating shear thinning behavior, while the k index, representing viscosity at a shear rate of 1 $s^{-1}$, increased with higher xanthan gum content. The tan(delta) values were higher for alginate-rich formulations but remained below 1, suggesting a predominant elastic component in xanthan-rich hydrogels. Better viscosity recovery was observed for xanthan-rich formulations, well aligned with their viscoelastic nature and enhanced elastic properties. These findings indicate that xanthan-rich hydrogels are more suitable for the intended applications. Future work will focus on biological compatibility studies and 3D bioprinting tests to optimize printing parameters. This research contributes to advance hydrogel development for tissue engineering and cultivated meat production.

**Keywords**: Tissue Engineering. Regenerative Medicine. Cultured meat. Hydrogel. Rheology.

## 1 INTRODUCTION

The formulation of hydrogels for regenerative medicine and cellular agriculture applications is a rapidly advancing field that holds significant promise for both medical therapies and sustainable food production. Hydrogels are versatile materials that can serve as bioinks for 3D bioprinting in tissue engineering and as injectable gels for the delivery of therapeutic molecules. Their ability to mimic the natural extracellular matrix (ECM) and support cell proliferation and differentiation makes them ideal candidates for these applications. In regenerative medicine, hydrogels are used to create scaffolds that facilitate tissue regeneration, while in cellular agriculture, they provide the structural support necessary for cell proliferation[1,2].

For hydrogels to be effective in these varied applications, they must meet several critical requirements. In 3D bioprinting, hydrogels need to exhibit appropriate rheological properties, such as shear-thinning behavior, to ensure smooth extrusion and precise deposition. The hydrogels must also present rapid gelation kinetics and sufficient mechanical strength to maintain the integrity of the printed structures. As injectable gels, they require biocompatibility, tunable degradation rates, and ability to release encapsulated therapeutic agents in a controlled manner. For both regenerative medicine and cellular agriculture, hydrogels must support cell adhesion, proliferation, and differentiation to promote the formation of functional tissues [1,3].

Understanding the rheological properties of hydrogels is crucial for optimizing their performance in these applications. Rheology provides insights into the flow behavior and mechanical properties of hydrogels, which directly impact their printability and injectability. For bioprinting, it is essential that hydrogels demonstrate shear-thinning behavior, allowing them to flow easily through the printing nozzle under stress and then quickly recover their structure post-deposition. For injectable applications, the viscosity of the hydrogel influences the ease of administration and the subsequent stability and performance of the gel within the body. Comprehensive rheological analysis ensures that the hydrogels possess the necessary properties for both precise printing and effective therapeutic delivery, if required [4].

In this study, we focus on formulating hydrogels using xanthan gum, alginate, and gelatin, leveraging their unique properties to address the requirements of regenerative medicine and cellular agriculture. Xanthan gum, known for its excellent viscoelastic properties and shear-thinning behavior, offers versatility in applications requiring precise material deposition and controlled release of encapsulated agents. Alginate, derived from brown algae, exhibits rapid gelation in the presence of divalent cations, making it suitable for fabricating stable scaffolds and injectable gels. Gelatin, derived from collagen, enhances cell adhesion and proliferation through its bioactive peptide sequences, contributing to tissue regeneration and cellular growth [2,5,6].

By combining xanthan gum, alginate, and gelatin, we aim to develop hydrogels with tailored properties suitable for a wide range of biomedical and agricultural applications. These hydrogels hold promise as versatile platforms for tissue engineering, drug delivery, and cultured meat production, contributing to advancements in regenerative medicine and sustainable food technologies.

## 2 MATERIAL & METHODS

Materials used to produce and crosslink the hydrogels: Alginate, gelatin, phosphate buffered saline, and calcium chloride were purchased from Sigma-Aldrich, and xanthan gum was obtained from CPKelco. Distilled water was used throughout the work.

Preparation of the hydrogels: Xanthan gum and alginate solutions were prepared in PBS. For complete dissolution of the polysaccharides and to obtain homogeneous solutions, the materials were mechanically stirred at 600 rpm for 30 min at room temperature. Then, the alginate solution was mixed with the xanthan solution at a 2:1, 1:1 or 1:2 polymer mass ratio. In parallel, a gelatin solution was prepared in PBS under mechanical stirring at 37°C. The gelatin solution was then mixed with the different alginate-xanthan solutions to obtain the AXG-2:1; AXG-1:1 and AXG-1:2 formulations. The resulting hydrogels, with a final concentration of 6% (w/v), were sterilized by autoclaving. Afterwards, the hydrogels were placed in syringes of up to 5 mL, centrifuged at 3000 rpm for 5 min to remove bubbles, and stored at 4°C. Samples were removed from refrigeration before the tests and all tests were performed at room temperature.

Qualitative analysis of flow behavior and crosslinking test: Qualitative analysis of the ability of homogeneous filament formation and hydrogel flow capacity were performed by syringe extrusion followed by microscopic evaluation and tube inversion test. The assessment of crosslinking time was performed using 100 mM $CaCl_2$.

Quantitative analysis of rheological behavior: The assessment of the rheological properties of the hydrogels was carried out in triplicates using an Anton-Paar MCR-102 modular compact rheometer, with a 50 mm diameter cone-plate geometry. The viscosity profile was analyzed at 25ºC with shear rates varying from 0,1 $s^{-1}$ to 3.000 $s^{-1}$. To calculate the n and k indices, the viscosity versus shear rate curve was fitted to the power-law model:

$$\eta = k\gamma^{n-1} \quad (1)$$

For frequency sweep measurements, the angular frequency was varied from 0.1 to 300 rad.$s^{-1}$. The percentage of deformation was maintained within the linear viscoelastic range (LVE). The damping factor, also called tan(delta), is given by the ratio of G'' (viscous modulus) to G' (elastic modulus). From the G' and G'' versus frequency curves, the maximum, average, and minimum values of tan(delta) were determined.

Three-interval thixotropy (3ITT) was performed to analyze the viscosity recovery of the hydrogel. During the initial rest interval, a low shear rate of 1 $s^{-1}$ was applied for 25 seconds. Then, the shear rate was increased to 100 $s^{-1}$ for 50 seconds and finally decreased back to 1 $s^{-1}$ for 200 seconds. The viscosity recovery was determined by the equation:

$$\textit{Recovery (\%)} = \textit{(final viscosity / initial viscosity)} \times 100 \quad (2)$$

## 3 RESULTS & DISCUSSION

Three formulations of alginate-xanthan-gelatin (AXG) hydrogels were tested: AXG-2:1, AXG-1:1, and AXG-1:2. In terms of polysaccharide content, AXG-2:1 contained more alginate, AXG-1:1 had equal mass proportions of alginate and xanthan, and AXG-1:2 had more xanthan gum. Qualitative and quantitative rheological analyses were performed.

Filament extrusion tests are crucial for evaluating the suitability of hydrogels for both 3D printing and injectable gel applications. In 3D printing, the ability to extrude a continuous, homogeneous filament is essential for constructing precise and stable structures. For injectable gels, extrusion performance indicates how easily the material can be delivered through a syringe without clogging or inconsistency. All AXG formulations extruded homogeneous filaments. As the proportion of xanthan increased, the hydrogel spread less on the deposition surface, indicating better shape retention. This suggests that xanthan gum enhances the structural integrity of the extruded filaments, making the hydrogel more suitable for applications requiring precise form retention.

The tube inversion test evaluates the gel's structural stability and resistance to flow, which is important for both 3D printing and injectable gel applications. A stable hydrogel that does not flow ensures accurate layer deposition in 3D printing and maintains its position when injected into a specific site. All AXG formulations showed no flow when the tube was inverted for up to 15 min, indicating excellent structural stability.

The gelation test measures the time required for the hydrogel to form a stable network upon exposure to a crosslinking agent, in this case, 100 mM $CaCl_2$. Rapid and consistent gelation is crucial for both 3D printing and injectable gels, as it affects the material's ability to form and retain structures immediately after deposition or injection. All formulations gelled within 30 min, demonstrating suitable gelation time for practical applications.

Rheological testing provides detailed insights into the hydrogel's flow behavior and mechanical properties, essential for tailoring materials for specific applications. The n index calculated from the viscosity x shear rate measurements was always lower than 1, suggesting a shear-thinning behavior for all formulations. The k index, representing viscosity at a shear rate of 1 $s^{-1}$, was higher in formulations with more xanthan, indicating greater viscosity in this case.

The damping factor, tan(delta), calculated as G'' (viscous modulus) divided by G' (elastic modulus), was obtained from the frequency sweep test. Tan(delta) was higher for formulations with more alginate but always remained below 1. This indicates that the elastic component was more significant in xanthan-rich formulations, contributing to their better structural integrity.

Three-interval thixotropy (3ITT) tests evaluated the hydrogel's ability to recover viscosity after shear stress. The viscosity recovery percentage was higher in xanthan-rich formulations, agreeing with previous observations of increased pseudoplasticity and elastic contribution in these formulations.

## 4 CONCLUSION

This study comprehensively evaluated AXG hydrogel formulations, demonstrating that those with higher xanthan content (AXG-1:2) exhibit superior structural integrity, shape retention, and viscoelastic properties. The findings collectively indicate that xanthan significantly enhances the viscoelastic properties, structural stability, and shape retention of the hydrogels, making them more suitable for both 3D printing and injectable applications in regenerative medicine and cellular agriculture.

Further tests are necessary to assess the biological compatibility of these formulations and to determine the optimal balance between mechanical properties and biocompatibility. Future studies will focus on *in vitro* evaluations to ensure that these hydrogels support cell viability, proliferation, and differentiation, crucial for their successful application in tissue engineering and cultivated meat production. Additionally, we will conduct 3D bioprinting tests to optimize printing parameters such as flow rate, print speed, and needle gauge for each formulation. These tests will help refine the hydrogel formulations to achieve the best possible printing quality and structural fidelity, ensuring their practicality and effectiveness.

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