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Rheological characterisation and printability assessment of an optimised bioink for extrusion-based 3D bioprinting

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ABSTRACT

The performance of extrusion-based 3D bioprinting depends critically on the rheology of bioinks, which must balance printability with post-deposition structural fidelity. Here we present a statistically optimised, human-compatible hydrogel formulation (hyaluronic acid, sodium alginate, Dextran-40), originally identified *via* Design of Experiment (DoE) methodology for target viscosity, and now subjected to comprehensive rheological validation. Flow curve analysis confirms the bioink's shear-thinning profile, supporting its suitability for extrusion. Oscillatory amplitude and frequency sweep tests reveal a stable viscoelastic response within the linear viscoelastic region. In combination with pronounced shear-thinning behaviour and rapid thixotropic recovery, this supports the bioink's ability to maintain structural integrity following deposition. A three-stage thixotropy test demonstrates rapid viscosity recovery following high shear, while temperature ramp testing shows expected increases in viscosity as temperature decreases, with no gelation observed in the printing-relevant range. Collectively, these findings validate the formulation's suitability for cell-laden printing applications and offer a reproducible rheological benchmark for future bioink development in soft tissue engineering.

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KEYWORDS
Rheology; bioinks; 3D bioprinting; extrusion printing; shear-thinning; thixotropy; viscoelastic

Introduction

The mechanical properties of biomaterials, such as hydrogels and bioinks, play a crucial role in the establishment of biomechanical interactions between cells and extracellular matrix which, in turn, influences the expression of cellular genotypes and the ensuing phenotypes [1,2].

Rheology is the study of how materials deform and flow under an applied force, and it is critical in assessing bioinks for 3D bioprinting; bioinks, composed of cells and biocompatible materials, must have precise rheological properties to ensure their printability, stability, and functionality [3–8]. Various types of forces can be used to obtain significant rheological data on the material examined.

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This article has been corrected with minor changes. These changes do not impact the academic content of the article.

Measurement of viscosity is a vital test for material characterisation of a fluid. Viscosity is defined as the measure of a material's resistance to flow when subjected to an applied stress [9]. A viscosity test involves examining how the viscosity of a material, e.g. a bioink, changes with varying shear rates. For bioinks to be effective in extrusion-based 3D bioprinting, the bioinks should exhibit shear-thinning behaviour, meaning their viscosity should decrease as the shear rate increases [6,10]. This property ensures that the bioink can be easily extruded through the printing nozzle, allowing for smooth printing without excessive force. When the bioink is too viscous, it can clog the nozzle, while a bioink that is too thin may lose its shape once deposited. After extrusion, the material must quickly regain higher viscosity to maintain the printed structure's shape [10,11].

Oscillatory rheology is another critical test for bioink characterisation [4,11]. Here oscillations of stress or strain are applied to a material to measure its viscoelastic properties. Two key parameters that are measured during these tests include the storage modulus (G′), representing the elastic, solid-like behaviour of the material, and the loss modulus (G′′), representing the viscous, liquid-like behaviour. Elastic behaviour is the material's ability to return to its original shape after a stress or strain is removed, whereas viscous behaviour is the material's ability to resist flow and deform gradually over time under stress. An elastic material stores and recovers energy, bouncing back to its original state when the applied stress is relieved. The balance between these two moduli is important for bioink performance. During extrusion, bioinks should behave more like flowing liquids, but after deposition, they should exhibit more solid-like characteristics to maintain structural integrity. The point at which the storage modulus surpasses the loss modulus indicates when the bioink transitions from a more fluid-like to a more solid-like state, which is crucial for solidifying the printed form [11,12].

Thixotropy testing measures how a material's viscosity changes over time under an applied shear force or stress. A thixotropic bioink thins under shear force (e.g. during extrusion) but quickly recovers viscosity once the applied force is removed. This recovery is essential to ensure the material doesn't collapse or spread after printing [13,14]. A bioink that recovers too slowly may lose its intended shape, whereas one that recovers quickly can maintain precise geometries, which is important when printing complex tissue structures.

Temperature-dependent rheology [13] is critical for bioinks, especially for those that are thermosensitive. During temperature sweep tests, the bioink's rheological properties are measured during changing temperature. Bioinks can either solidify or gel at physiological temperatures [15], and it is necessary to understand a bioink's behaviour during the printing process and essentially in the target physiological environment, such as the human body. When a bioink gels too quickly it can end up solidifying prematurely, whereas when it gels too slowly it can fail to form stable structures in the desired environment [16]. The temperature focused tests ensure that the bioink remains in a liquid state during printing and then solidifies appropriately at the target temperature, which is vital for ensuring stable and functional tissue constructs.

Overall, rheological tests offer critical insights into the flow, deformation, and recovery characteristics of bioinks, enabling their optimisation for extrusion-based 3D

bioprinting. Previous studies have systematically characterised the rheological behaviour of simple hydrogel systems [17–19], demonstrating key structure–modulus relationships. This study builds directly on our previous work (Upton et al. 2025) [20], in which a Design of Experiment (DoE) approach was used to formulate and optimise a bioink composed of hyaluronic acid, sodium alginate, and Dextran-40, all of which are extensively used in tissue engineering due to their well-established biocompatibility [21,22]. To our knowledge, this is the first study to comprehensively evaluate the rheological behaviour - including shear-thinning, linear viscoelastic region (LVR), thixotropy, and thermal response - of an optimised bioink formulation previously optimised *via* statistical design. The optimised formulation described forms the basis of the current work, which investigates the detailed rheological performance of the bioink under conditions relevant to extrusion-based 3D bioprinting and provides a comprehensive evaluation of the bioink's printability and suitability for cell-laden applications.

Methods and material

Materials

The optimised bioink was prepared using materials and concentrations as described in Upton, A. et al. (2025) [20]. The concentrations were selected following statistical optimisation using Design of Experiment (DoE) methodology and aimed at achieving a target viscosity suitable for extrusion-based 3D bioprinting. Specifically, the bioink was prepared using 1.25% w/v hyaluronic acid (Biosynth, UK – Molecular Weight: 1–2 million Daltons), 2.52% w/v sodium alginate (Thermo-Scientific, UK – Molecular Weight: 12000–40000 Daltons), and 0.5% w/v Dextran-40 (Thermo Scientific, UK) dissolved in phenol-free DMEM (Gibco, UK) and supplemented with 5% human platelet lysate (HPL) (Stem Cell Technologies, UK).

Bioink preparation

Samples were prepared as previously described in Upton, A. et al. (2025) [20]. Specifically, each sample component was weighed out to the desired concentration (w/v%) and UV-sterilised in a UV-clave for 15 min. Final samples were manually mixed between two luer-lock syringes for 10 min, ensuring complete homogenisation. An Invitrogen M5000 optical microscope was used to confirm absence of phase separation and clumping, thereby verifying the homogenisation of the sample (visualisation under brightfield at x10 magnification).

Rheological characterisation

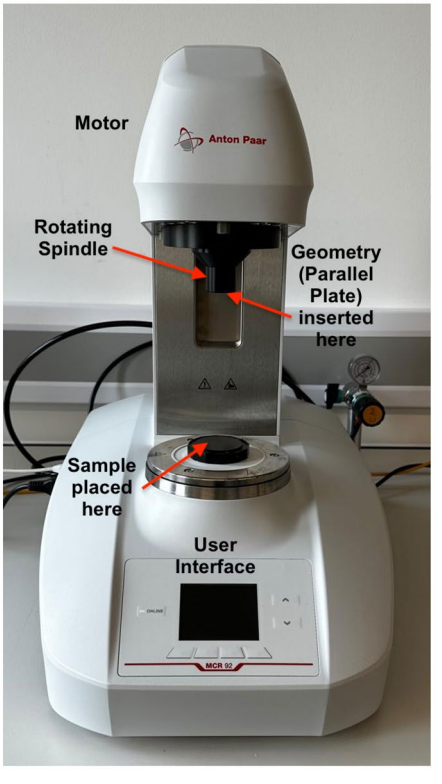
Rheometer

An Anton Paar MCR 92 rheometer was used to carry out rheological testing and the resulting data was collected through the RheoCompass 1.30 software. The methodology used was adapted from Cavallo et al. [23] and is as follows: a parallel plate geometry of 25 mm was employed with 1 ml of the sample added using a syringe to

the rheometer for testing. The geometry was lowered to a zero-gap of 1 mm and, *via* use of a plastic spatula, excess sample was carefully removed to ensure consistent sample thickness. Samples were allowed to equilibrate for 60 s at the test temperature prior to testing, to ensure thermal stabilisation. Details of tests performed are described below. All tests were carried out using independently prepared bioink samples for each test. All samples were measured at a constant temperature of either 25 °C or 37 °C, depending on the test, with the exception of the temperature ramp test. An image of the rheometer used can be seen in Figure 1.

Flow curve test

This rotational test was applied to determine the bioink's viscous flow behaviour when subjected to a pre-set increasing ramp of shear rate. The viscosity was measured for a linear ramp of shear rate increasing from 1 to 100s^{-1}.



Rheometer

Anton Paar MCR 92 rheometer used for experiments presented in this paper. A parallel plate 25mm geometry was used for all runs.

Figure 1. Rheometer.
Anton Paar MCR 92 rheometer used for experiments presented in this paper. A parallel plate 25mm geometry was used for all runs.

Amplitude sweep test

This oscillatory test was used to identify the bioink's linear viscoelastic region (LVR). The test was programmed with a logarithmic shear strain ramp from 0.0001% – 10%, to observe low and high strain regions, with a constant pre-set angular frequency of 10 rad/s. The loss modulus (G″) and storage modulus (G′) were measured and collected automatically as set by the instrument.

Frequency sweep test

This oscillatory test was used to define the time-dependent behaviour of the bioink in the non-destructive deformation range which lies within the previously identified LVR (see amplitude sweep test). The oscillating shear strain was typically set at 1%, with the angular frequency ranging from 1 to 100 rad/s. The loss modulus (G″) and storage modulus (G′) were measured and were collected automatically as set by the instrument.

Thixotropy test

This test was employed to determine the bioink's time-dependent thixotropic behaviour, i.e. to evaluate its ability to recover its structure following shear-induced breakdown. The rate and degree of viscosity recovery are of importance here. The bioink was subjected to 3 intervals. The first interval and last interval were oscillatory in nature, with a pre-set shear strain of 1% and pre-set frequency of 1 Hz, measuring the complex viscosity (η^*). These parameters were selected based on the aforementioned amplitude and frequency sweep tests, confirming that the strain fell within the material's linear viscoelastic region (LVR), thereby avoiding structural disruption during measurements. The second interval was a rotational interval with a pre-set shear rate of 1000s^{-1}, measuring the apparent viscosity (η) of the bioink. The first interval duration of 120s established baseline structure. The second interval duration of 30s simulated extrusion conditions, whereas the third interval of 400s monitored the recovery of the bioink's viscosity, thus providing insight into its post-printing ability to maintain its structure.

Temperature ramp test

This test was designed to evaluate the bioink's temperature-dependent flow behaviour across a biologically relevant range. The bioink was loaded onto the rheometer and the initial temperature was set at 40 °C for 1 min. The sample was then subjected to constant pre-shearing for 3 min at shear rate of 10s^{-1} to standardise flow. A linear temperature ramp was then applied, decreasing from 40 °C to 0 °C, while at a constant shear rate of 80s^{-1}. During this phase, the apparent viscosity (η) was continuously recorded under constant shear conditions, with data points collected every 0.5 °C, resulting in 41 measurements.

Results

Shear-thinning behaviour and flow curve analysis

Understanding the relationship between shear stress (τ) and shear rate ($\dot{\gamma}$) is crucial in determining the flow behaviour of the bioink [4]. The bioink was subjected to

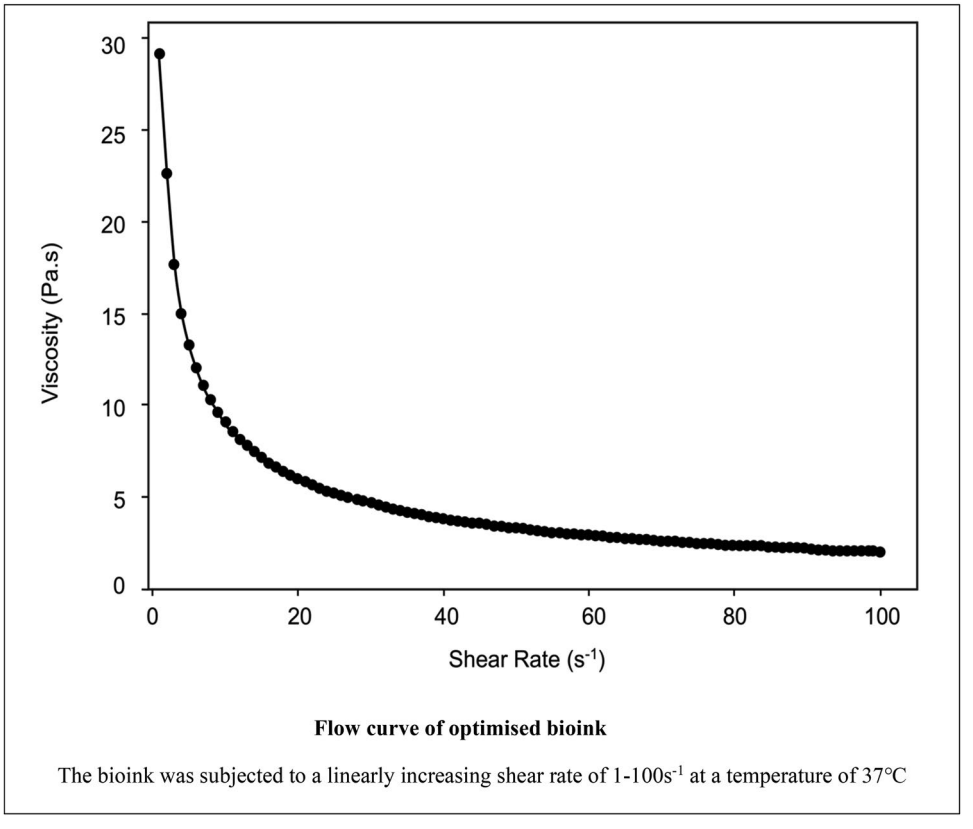


Figure 2. Flow curve of optimised bioink.
The bioink was subjected to a linearly increasing shear rate of 1-100 s^{-1} at a temperature of 37 °C

an increasing shear rate from 1 to 100 s^{-1}, at a constant temperature of 37 °C, chosen to replicate the physiological human body temperature. Figure 2 demonstrates the resulting flow curve. The viscosity of the bioink decreased from approximately 29.5 Pa·s at a shear rate of 1 s^{-1} to around 2.3 Pa·s at 100 s^{-1}. The concave curve seen is typical of non-Newtonian fluid exhibiting shear-thinning flow behaviour.

Viscoelastic properties from amplitude and frequency sweep tests

Amplitude sweep analysis was performed to determine the linear viscoelastic region (LVR) of the bioink. Both the storage modulus (G′) and loss modulus (G″) were measured across increasing shear strain, and the results are presented in Figure 3. The LVR lies within 0.0001% – 0.5% of the shear strain and in this region G′ remained below G″, indicating that the viscous (liquid-like) behaviour dominated over that of the elastic (solid-like). This behaviour confirms that the material remains within a viscoelastic regime under low deformation. Beyond this range, a decline in the moduli was observed, indicating the onset of structural breakdown and the limit of the linear viscoelastic region. A comparable trend was observed when the data were represented as a function of shear stress, with the moduli remaining stable across the LVR and a decline occurring at high amplitudes, just below 100%.

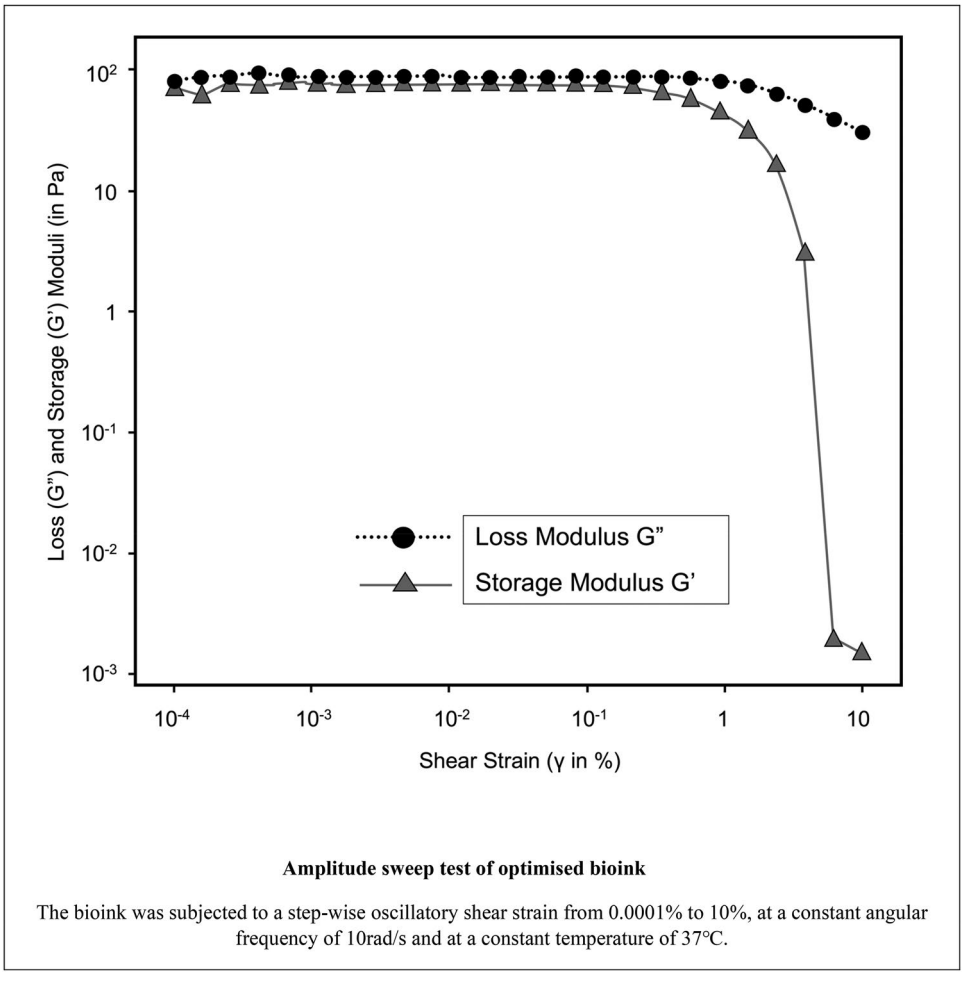


Figure 3. Amplitude sweep test of optimised bioink.
The bioink was subjected to a step-wise oscillatory shear strain from 0.0001% to 10%, at a constant angular frequency of 10rad/s and at a constant temperature of 37 °C.

To further assess the viscoelastic properties under oscillatory deformation, a frequency sweep test was conducted, as shown in Figure 4. The frequency sweep test provides insight into the viscoelastic behaviour of the hydrogel network under oscillatory deformation. Once again, what was observed across the tested angular frequency range was the fact the storage modulus (G′) remained consistently lower than the loss modulus (G′′), indicating that the material behaves predominantly as a viscous-dominated viscoelastic material.

Specifically, within the angular frequency range of 1 to approximately 90 rad/s, the loss modulus G′′ (viscous behaviour) remained consistently greater than the storage modulus G′ (elastic behaviour), once again indicating that the material behaves predominantly as a viscous-dominated viscoelastic material. At lower angular frequencies (below ~30 rad/s), both moduli rise slightly, after which G′ plateaus whereas G′′ continues to rise across the measured range. This behaviour is consistent with a viscoelastic material exhibiting frequency-dependent viscous behaviour. At higher

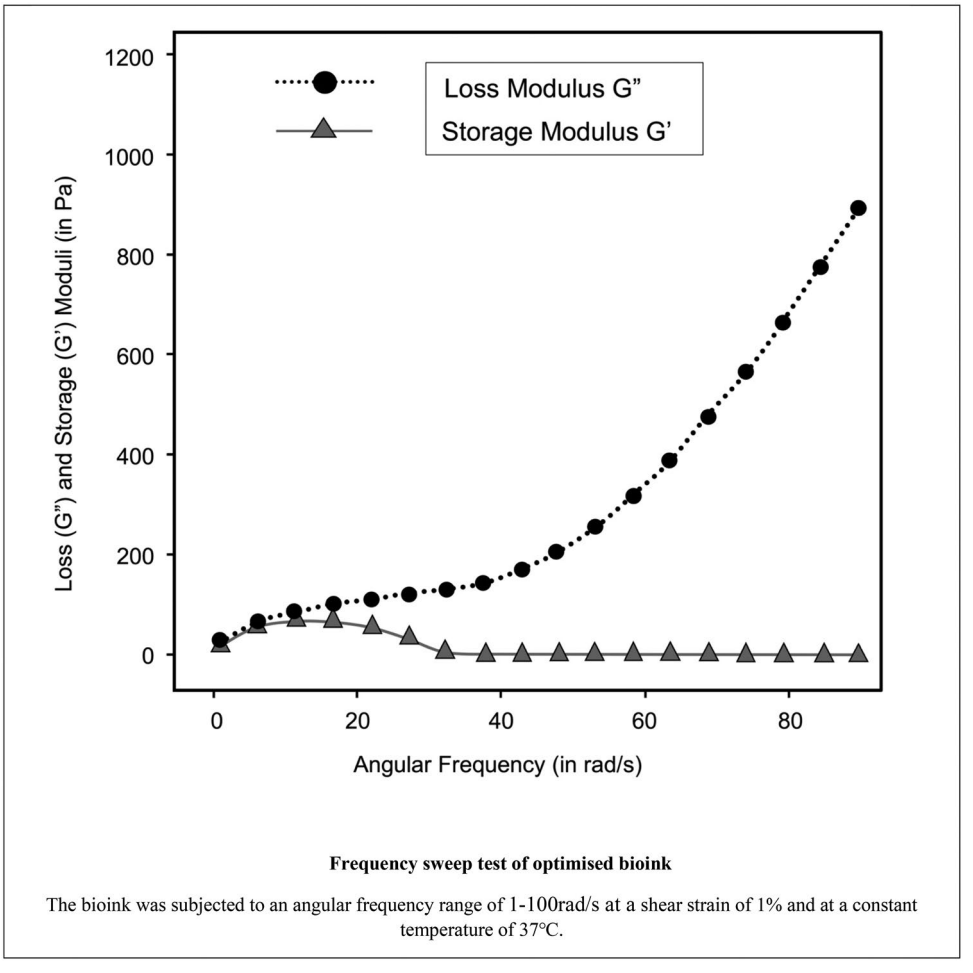


Figure 4. Frequency sweep test of optimised bioink.
The bioink was subjected to an angular frequency range of 1-100rad/s at a shear strain of 1% and at a constant temperature of 37 °C.

frequencies, the response reflects reduced relaxation of the internal structure on shorter timescales. This is observed as a widening separation between G′ and G″, indicating a stronger viscous response at higher frequencies.

Thixotropic recovery

The thixotropy test was conducted to assess the bioink's ability to recover its viscosity following shear-induced thinning. The outcome of this test is shown on Figure 5. The 3 interval stages are separated *via* two vertical dashed grey lines. The first and last intervals subject the bioink to a low frequency of 1 Hz, with the first interval lasting 120s and the last lasting 400s. The intermediate interval includes a high rotational shear rate of $1000s^{-1}$ applied for 30s.

During the first interval the bioink experiences a slight increase in viscosity, most likely due to the internal network reforming under the low oscillatory shear being

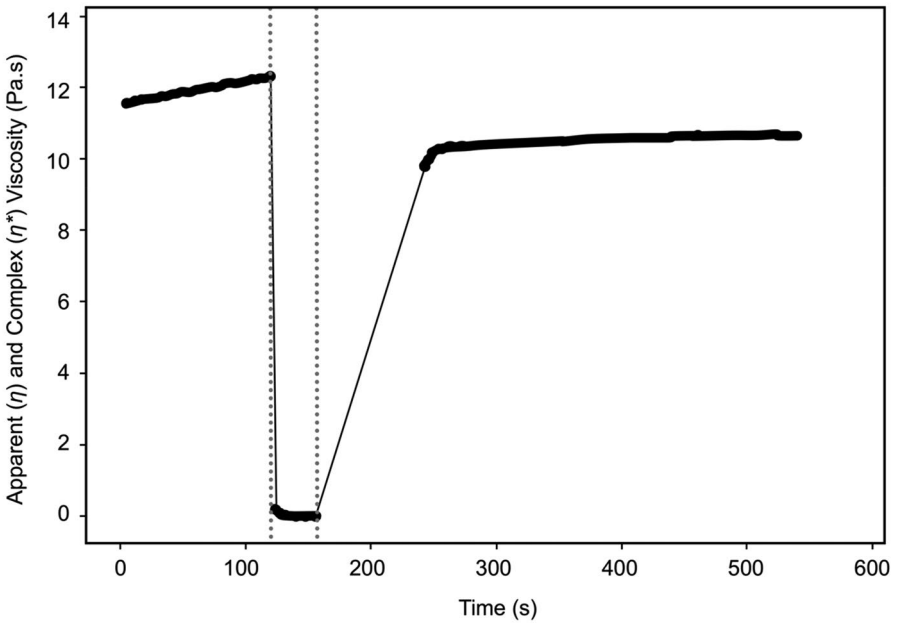


Figure 5. Thixotropic test on the optimised bioink.
The bioink was subjected to 3 intervals: the first interval of 120s subjected the bioink to a low, oscillatory force of frequency 1Hz. The second interval of 30s subjected the bioink to a high rotational force of 1000s^{-1}, whereas the third interval of 400s subjected the bioink to the initial low frequency of 1Hz. The vertical dashed lines are used to separate interval stages. In intervals 1 and 3 the complex viscosity (Pa·s), was measured and in interval 2 the apparent viscosity (Pa·s) was measured.

applied. In the second interval the bioink's viscosity rapidly decreases and remains low, as expected for the shear-thinning material. The last interval sees the removal of the high rotational shear rate whereby the bioink begins to immediately, and rapidly, recover its viscosity, reaching approximately 90% of its original value within 40s.

Temperature-dependent rheology

The bioink was subjected to a decreasing linear temperature ramp test from 40 °C to 0°C whilst under a constant shear rate of 80s^{-1}. The resulting graph, shown in Figure 6, reveals that as the temperature decreases, the apparent viscosity (η) of the bioink increases gradually. At higher temperatures the bioink's viscosity remains relatively constant, increasing only slightly as temperature decreases, however at lower temperatures the increase in viscosity is more significant, with the decrease in temperature now appearing to be linearly proportional to the increase in viscosity. At no point

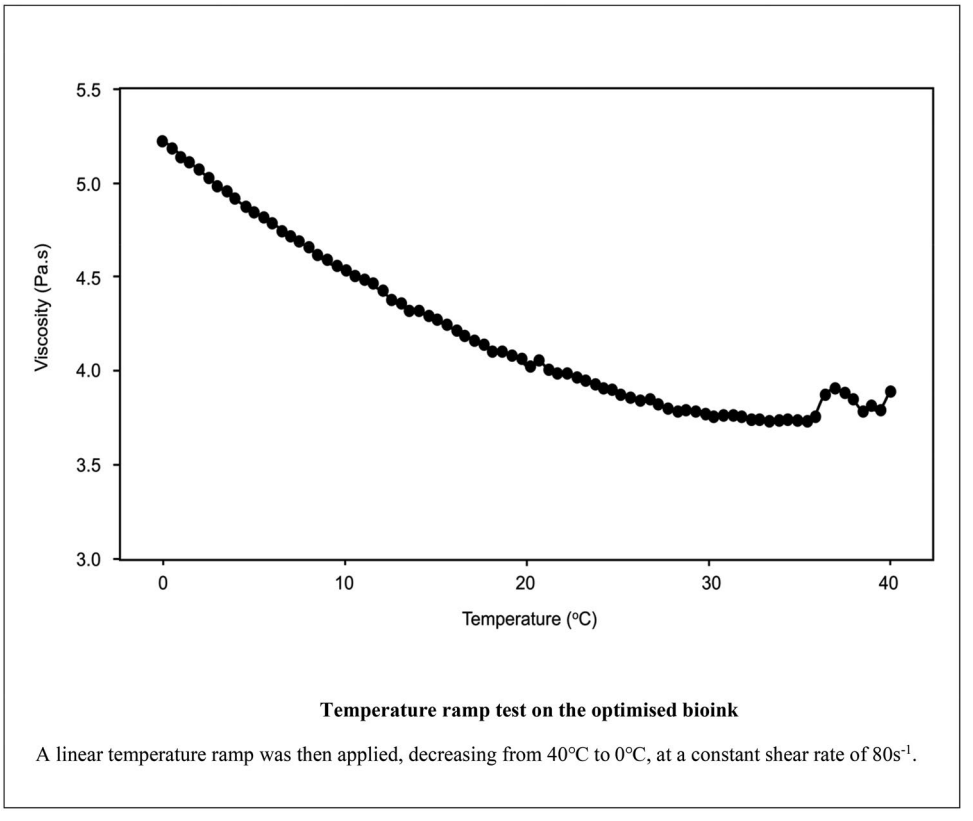


Figure 6. Temperature ramp test on the optimised bioink.
A linear temperature ramp was then applied, decreasing from 40 °C to 0°C, at a constant shear rate of $80s^{-1}$.

was there a breakdown in the viscosity value, indicating the bioink remained processable across a biologically relevant temperature range. This behaviour ensures stability during printing and allows post-printing solidification at physiological temperatures. Observable in the figure is the sudden change in viscosity at 38 °C, indicating a possible structural rearrangement within the hydrogel network.

Discussion

The rheological properties of bioinks play a fundamental role in their behaviour during extrusion-based 3D bioprinting. The ability of a bioink to flow smoothly through a nozzle while maintaining structural integrity after deposition depends on key factors such as shear-thinning behaviour, thixotropic recovery, temperature-dependent viscosity, and viscoelasticity [18].

The results obtained from this study provide valuable insights into the bioink's printability and mechanical stability, demonstrating its suitability for bioprinting applications. By analysing these properties, we can better understand how the bioink responds to different printing conditions and how it can be further refined for tissue engineering applications.

The flow curve results confirmed the bioink's shear-thinning behaviour, a critical property for extrusion-based 3D bioprinting. Shear-thinning occurs when viscosity decreases under increasing shear rate, allowing for smooth extrusion through the printing nozzle while preventing excessive mechanical stress on cells. This behaviour is particularly important in ensuring cell-laden bioinks can be extruded without requiring high extrusion pressures, which would lead to cell damage [24]. Bioinks lacking shear-thinning capabilities may require higher extrusion forces, which can cause nozzle clogging and impair cell viability due to excessive shear stress.

Conversely, an overly fluid bioink that does not recover viscosity post-extrusion is likely to produce printed structures with poor shape fidelity and structural collapse. As such, thixotropic behaviour - which describes a material's ability to regain viscosity after shear stress is removed - is another essential factor influencing bioprinting success [10].

Previous studies have proposed rheological criteria associated with good printability [12,14,25,26]. Paxton et al. [12] suggest that printable bioinks typically exhibit pronounced shear-thinning behaviour and rapid structural recovery (>80%). Gao et al. [26] highlighted the importance of viscoelastic properties in balancing extrusion and structural stability, noting that the relationship between storage and loss moduli influences bioink performance.

The optimised formulation examined in this study satisfies such key rheological criteria, exhibiting pronounced shear-thinning behaviour and rapid viscosity recovery. In addition, the bioink demonstrates a consistent viscoelastic response across the tested range. Collectively, these characteristics support the suitability of the formulation for extrusion-based applications and validate the effectiveness of the DoE-driven optimisation approach.

This study is, to the best of our knowledge, the first to provide a detailed report on the linear viscoelastic region and thixotropy recovery on the specific hyaluronic acid – sodium alginate – Dextran-40 bioink matrix. The thixotropic test results demonstrated the bioink experienced a significant viscosity drop during the high-shear phase, however it rapidly recovered once shear stress was removed, revealing an approximately 90% recovery within 40s. The ability to recover viscosity and do so rapidly ensures that once the bioink is extruded, it regains viscosity quickly enough to support subsequent layers in a multi-layered construct. In practical applications, a bioink with slow viscosity recovery may result in structures that spread or collapse before they can solidify adequately. On the other hand, a bioink with excessively fast recovery may not allow for adequate layer fusion, leading to weak interfaces between printed layers. The observed thixotropic recovery of the examined bioink indicates towards striking a balance between rapid viscosity regain and sufficient flowability, supporting both high-resolution printing and stable construct formation.

Temperature stability is another crucial parameter for bioinks. The temperature ramp test demonstrated a controlled increase in viscosity as the temperature decreased from 40 °C to 0 °C. This behaviour is beneficial for bioprinting applications, where bioinks are printed at slightly elevated temperatures but must solidify upon cooling to physiological conditions. Many hydrogel-based bioinks exhibit temperature-sensitive behaviour, undergoing network stabilisation at lower temperatures [15]. A well-controlled temperature response prevents premature gelation inside the print nozzle, which can cause clogging and hinder extrusion. Conversely, if the viscosity

remains too low at physiological temperatures, the bioink may fail to support complex structures, leading to construct deformation. The gradual viscosity increase observed in this study demonstrates that the bioink remains processable during printing while ensuring post-printing mechanical stability.

Although a change in viscosity was observed near 38 °C, the standard incubation temperature used for most cell culture is 37 °C, which lies slightly below the observed transition point, indicating that the hydrogel network remains within a stable rheological regime under typical biological conditions. For 3D printing of hydrogels as scaffolding material, additional stabilisation strategies - such as ionic crosslinking of alginate with calcium ions - are often employed to further reinforce the structural integrity of the scaffold [27].

The viscoelastic nature of the bioink is essential for determining its mechanical performance post-printing. Oscillatory rheology tests, including amplitude and frequency sweeps, provided valuable insights into the bioink's viscoelasticity. The amplitude sweep test demonstrated that within the linear viscoelastic region both storage and loss moduli remained stable, indicating that the bioink maintains structural integrity under low deformation. This viscoelastic behaviour is important for maintaining the shape of printed constructs, preventing collapse under gravity, and ensuring the mechanical stability of bioprinted structures [10].

The frequency sweep test further confirmed the material's viscoelastic stability over a range of oscillation frequencies, demonstrating a consistent viscoelastic response across the tested range. This behaviour confirms that the bioink can withstand mechanical stresses during handling and post-printing maturation. Bioinks that lack sufficient elasticity may sag or flow after deposition, compromising structural fidelity. However, excessive rigidity can be detrimental as well, as it may impede cellular migration and proliferation [28]. The balanced viscoelastic properties observed in this study suggest that the bioink provides sufficient mechanical support while maintaining the necessary flexibility for tissue maturation.

The rheological characterisation presented in this study builds directly upon our previous optimisation work [20], in which a systematic Design of Experiment (DoE) approach was used to identify a bioink formulation with target viscosity and robust manufacturing reproducibility. By applying a detailed suite of rheological tests to the optimised formulation, this study validates not only the statistical optimisation process but also confirms that the bioink exhibits the critical behaviours required for extrusion-based 3D printing, including shear-thinning, structural recovery, and thermal responsiveness.

This study was focused on the rheological behaviour of the bioink in its hydrated condition, as this is relevant to extrusion-based bioprinting. Drying behaviour under varying environmental conditions was not examined as this is specifically relevant to post-print scaffold stability or storage applications and, as such, beyond the scope of the current rheological investigation.

Conclusion

This work represents the second phase of our ongoing development of human-based bioinks for tissue engineering applications. This rheological characterisation of a

previously statistically optimised bioink confirms its suitability for extrusion-based 3D bioprinting. Its shear-thinning behaviour enables smooth extrusion, while rapid thixotropic recovery maintains structural integrity post-printing. The temperature-dependent viscosity ensures processability and stability at physiological conditions, and its viscoelastic properties provide mechanical support without compromising flexibility for cellular activity.

In our previous study this bioink was identified as an optimised formulation through combination of Design of Experiment (DoE) methodology and viscosity measurements [20], whereas the present study confirms that the bioink meets not only the target viscosity but also additional rheological criteria essential for extrusion-based printing. Together, these two studies establish a complete optimisation-to-validation procedure, from statistical formulation to print-ready mechanical assessment. This is a rarely reported and valuable blueprint for bioink development, offering a complete pipeline from formulation design to functional performance evaluation.

Overall, the combination of shear-thinning flow behaviour, rapid thixotropic recovery, and consistent viscoelastic response indicates that the bioink possesses key rheological characteristics required for extrusion-based processing while supporting structural stability after deposition.

Future work will involve incorporating live cells into the optimised matrix to evaluate how cellular content influences rheological properties, printability, and biological performance.

Author contributions

CRediT: **Alice Upton**: Investigation, Writing – original draft; **Athina Mylona**: Investigation, Supervision, Writing – review & editing; **Georgina Zimbitas**: Investigation, Supervision, Writing – review & editing.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Ethical approval

Not applicable (no human tissue used in the experiments presented in this paper).

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Data availability statement

Data for experimental runs are presented in this paper. The composition of the examined bioink was determined through the use of Design of Experiment (DoE) methodology and viscosity measurements, as presented in the paper Upton et al. [20], whereby Minitab® 21 was used to create DoE.

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